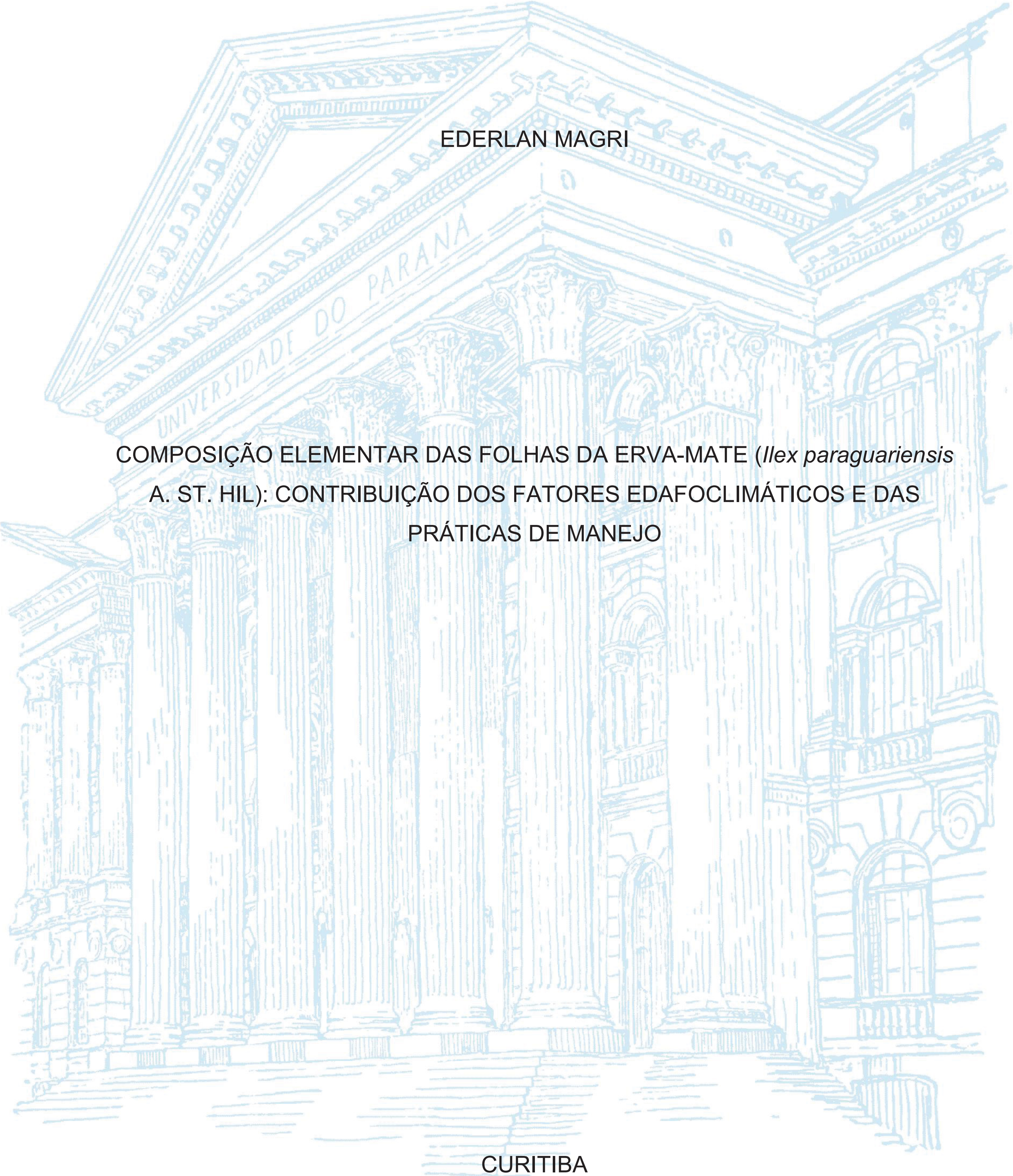
UNIVERSIDADE FEDERAL DO PARANÁ



EDERLAN MAGRI

COMPOSIÇÃO ELEMENTAR DAS FOLHAS DA ERVA-MATE (*Ilex paraguariensis* A. ST. HIL): CONTRIBUIÇÃO DOS FATORES EDAFOCLIMÁTICOS E DAS PRÁTICAS DE MANEJO

CURITIBA
2021

EDERLAN MAGRI

COMPOSIÇÃO ELEMENTAR DAS FOLHAS DA ERVA-MATE (*Ilex paraguariensis* A. ST. HIL): CONTRIBUIÇÃO DOS FATORES EDAFOCLIMÁTICOS E DAS PRÁTICAS DE MANEJO

Tese apresentada ao curso de Pós-Graduação em Ciência do Solo, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Ciência do Solo.

Orientador: Dr. Antônio Carlos Vargas Motta

Coorientadores: Dra. Alice Teresa Valduga

Dr. Julierme Zimmer Barbosa

CURITIBA
2021

Magri, Ederlan
Elementary composition of yerma mate leaves (*Ilex paraguariensis* A. St. Hil): contribution of edafoclimatic factors and management practices = Composição elementar das folhas da erva-mate (*Ilex paraguariensis* A. St. Hil): contribuição dos fatores edafoclimáticos e das práticas de manejo. / Ederlan Magri. - Curitiba, 2021.

Tese (Doutorado) - Universidade Federal do Paraná. Setor de Ciências Agrárias, Programa de Pós-Graduação em Ciência do Solo.
Orientação: Antônio Carlos Vargas Motta
Coorientação: Alice Teresa Valduga
Coorientação: Julierme Zimmer Barbosa

1. Erva-mate. 2. Cadmio. 3. Chumbo. I. Motta, Antônio Carlos Vargas. II. Valduga, Alice Teresa. III. Barbosa, Julierme Zimmer. IV. Título. V. Universidade Federal do Paraná.

Sistema de Bibliotecas/UFPR
Douglas Alex Jankoski – CRB 9/1167

MINISTÉRIO DA EDUCAÇÃO
SETOR DE CIENCIAS AGRARIAS
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO CIÊNCIA DO SOLO -
40001016014P4

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIA DO SOLO da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **EDERLAN MAGRI** intitulada: **COMPOSIÇÃO ELEMENTAR DAS FOLHAS DA ERVA-MATE (Ilex paraguariensis A. ST. HIL): CONTRIBUIÇÃO DOS FATORES EDAFOCLIMÁTICOS E DAS PRÁTICAS DE MANEJO**, sob orientação do Prof. Dr. ANTONIO CARLOS VARGAS MOTTA, que após terem inquirido o aluno e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 23 de Abril de 2021.

Assinatura Eletrônica
23/04/2021 15:12:37.0
ANTONIO CARLOS VARGAS MOTTA
Presidente da Banca Examinadora

Assinatura Eletrônica
23/04/2021 16:31:16.0
ELIZIANE LUIZA BENEDETTI
Avaliador Externo (INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA DE SANTA CATARINA)

Assinatura Eletrônica
23/04/2021 17:33:05.0
LEANDRO FLAVIO CARNEIRO
Avaliador Externo (UNIVERSIDADE FEDERAL DO PARANá- DPTO FITOTECNIA E FITOSSANITARISMO)

Assinatura Eletrônica
23/04/2021 17:34:25.0
JULIERME ZIMMER BARBOSA
Avaliador Externo (INSTITUTO FEDERAL SUDESTE DE MINAS GERAIS - CAMPUS BARBACENA)

Assinatura Eletrônica
26/04/2021 10:18:25.0
ALICE TERESA VALDUGA
Avaliador Externo (UNIV. REGIONAL INTEGRADA DO ALTO URUGUAI E DAS MISSÕES)

To my parents and brother:

Primo Antônio Magri
Silvana Fátima Zanlucchi Magri
Diego Antônio Magri

ACKNOWLEDGMENTS

I am grateful to the Federal University of Paraná, especially to the Graduate Program in Soil Sciences, for this opportunity for personal and professional advancement.

To my advisor, Antônio Carlos Vargas Motta, for his friendship, trust, and efforts to make me a better professional.

To my co-supervisor, Alice Tersa Valduga, who has always motivated and guided me on this academic journey.

To my co-supervisor, Julierme Zimmer Barbosa, for all the dedication and help provided during this period.

To Andréa Oliveira and her advisors, Iohanna and Patrícia, for their assistance in developing the methodology for quantification of Cd and Pb.

To the teachers of the PPG Sciences of the Soil, especially those who were most involved: Volnei Pauletti, Vander de Freitas Melo, André Carlos Auler and Jair Alves Dionizio.

To the technicians of the laboratories of the Department of Soil and Agricultural Engineering, especially Maria, Fabiana and Carla; and to the employees of the UFPR electron microscopy center for their assistance in the analyses.

To my postgraduate colleagues, especially Cleiton Frigo, Diego de Oliveira Rabel, Ricardo Ribeiro, Felipe Bratti, Kayo Alberto and Felipe Grabarski, for all the teaching and help provided.

To CAPES, for financing the teaching scholarship.

To the Mate Industry Syndicate of the state of Rio Grande do Sul, Santa Catarina and Paraná, and affiliated industries, for funding this research.

To the yerba mate industries, Barão Erva-mate and Teas, especially Sérgio and Ana Piccolo; and Erva-mate Ximango, especially to Fábio, for all the assistance provided.

To the Universidade Regional Integrada do Alto Uruguai e das Missões (URI) – Erechim campus, for the partnership in conducting this work.

To the members of the defense panel - Eliziane Luiza Benedetti, Leandro Flávio Carneiro, and Carlos Bruno Reissmann for their dedication in reading this thesis and for agreeing to participate in the conclusion of this academic journey.

To my friends, Eduardo Somensi and Cilmar Dalmaso, for all the exchange of knowledge during this important period.

To my friends, Eduardo Gugelmin and Henrique Santos, for their help with laboratory activities and experiments.

To my brother, Diego Magri and my sister-in-law, Katia Magri, for having welcomed me into their home at the beginning of this journey.

To my parents, Primo and Silvana Magri, for their strength and encouragement.

To my companion, Juliana Andrioli Ribeiro, for all the support provided and for always understanding.

To all, my sincere thanks.

RESUMO

As folhas da erva-mate (*Ilex paraguariensis* A. St. Hil) são exploradas há centenas de anos para a produção de bebidas de infusão, tradicionalmente consumidas na região austral da América do Sul. Esta espécie é cultivada em uma extensa área, com diferentes manejos e propriedades edafoclimáticas. Adicionalmente, a legislação local estabelece limites máximos para a presença de Cd (0,40 mg kg^{-1}) e Pb (0,60 mg kg^{-1}) no produto comercial da erva-mate. Assim, buscou-se investigar a contribuição dos fatores edafoclimáticos e do manejo na composição elementar das folhas de erva-mate, especialmente dos elementos potencialmente tóxicos Cd e Pb. Desta forma, esta tese está seccionada em quatro capítulos, onde objetivou-se: (i) investigar a presença de Cd e Pb nos principais polos produtores de erva-mate na América do Sul, por meio de 115 amostras tomadas no Brasil, Argentina e no Paraguai. Verificou-se que o Cd e Pb ocorrem naturalmente nas folhas da erva-mate em teores de até três vezes maior que os limites estabelecidos na legislação, sugerindo que estes limites necessitam ser revisados; (ii) Nos mesmos sítios descritos acima, buscou-se identificar a contribuição dos fatores edafoclimáticos e do sistema de cultivo (agroflorestas e monocultivos) na composição elementar das folhas de erva-mate. Verificou-se diferença nos teores de P e Mn entre os sistemas de cultivo, apresentando maior teor de Mn e menor de P nas amostras de agroflorestas em relação aos monocultivos. Foi constatado correlação negativa entre o Mn foliar e o pH do solo. Já o teor de B e Ca foliar mostrou ser dependente da temperatura média anual, com valores mais elevados nas regiões mais quentes. Adicionalmente, o teor de N foliar foi correlacionado com o teor de matéria orgânica do solo e com a altitude. Foi possível concluir que as condições de crescimento em que a erva-mate é submetida tem implicações na composição elementar de suas folhas, bem como na qualidade do produto gerado a partir delas; (iii) Buscou-se investigar a relação entre o uso de glifosato e adubação fosfatada na composição elementar de dois clones de erva-mate cultivados em dois solos com material de origem distintos (experimento com vasos), especialmente com relação aos elementos potencialmente tóxicos Cd e Pb. O uso de glifosato no controle de plantas indesejáveis prejudicou o desenvolvimento inicial da erva-mate, sendo potencializado na carência de P. A composição elementar das folhas da erva-mate também foi alterada com o uso de glifosato, dado o acréscimo nos teores de alguns elementos, incluindo pequenos acréscimos de Cd e Pb. No entanto, os efeitos observados no crescimento e na composição elementar foram distintos entre os cultivares e o tipo do solo. (iv) Foi avaliado a capacidade de acumulação de Mn nas folhas de erva-mate submetida a doses crescentes de Mn em dois solos com e sem correção da acidez (experimento em vasos). A erva-mate mostrou-se uma planta hiperacumuladora de Mn, atingindo teor de 13,452 mg kg^{-1} sem prejudicar o crescimento da planta. Foi constatado diferença no teor foliar de Mn de acordo com o material de origem do solo (basalto>arenito); e com a correção da acidez do solo (sem calagem>com calagem).

Palavras-chave: Composição elementar. Legislação. Cádmio. Chumbo. Manganês.

ABSTRACT

The leaves of yerba mate (*Ilex paraguariensis* A. St. Hil) have been exploited for hundreds of years in the production of infusion drinks, traditionally consumed in the southern region of South America. This species is grown extensively in areas with differing crop systems and edaphoclimatic properties. In addition, local legislation establishes maximum limits on the presence of Cd (0.40 mg kg^{-1}) and Pb (0.60 mg kg^{-1}) in the commercial production of yerba mate. This study investigated the contribution of edaphoclimatic factors and management on the elemental composition of yerba mate, with special consideration given to the potentially toxic elements, Cd and Pb. The thesis is divided into four chapters: (i) Investigating the presence of Cd and Pb in the main yerba mate production regions of South America. 115 samples were taken from Brazil, Argentina and Paraguay. Results showed that Cd and Pb occur naturally in the leaves of yerba mate at levels up to three times higher than the limit established in the legislation, suggesting that these limits need to be revised; (ii) Analyzing the contribution of edaphoclimatic factors and the cultivation system (agroforestry and plantations) to the elementary composition of yerba mate leaves, in the same geographical regions as described above. A difference in the concentrations of P and Mn was identified between the cultivation systems, with a higher content of Mn and a lower of P in the samples of agroforestry relative to monocultures. A negative correlation was found between leaf Mn and soil pH. The leaf B content was shown to be dependent on the average annual temperature, with higher values in the warmer regions. In addition, the leaf N content was correlated with the soil organic matter content and with altitude. It was concluded that the growing conditions in which the yerba mate are subjected have implications for the elemental composition of its leaves, as well as for the quality of the product generated from them; (iii) Investigating the relationship between the use of glyphosate and phosphate fertilization in the elemental composition of two yerba mate clones. During a potting experiment, these were grown in two types of soil of differing parent material, with special emphasis on the potentially toxic elements, Cd and Pb. The use of glyphosate in the control of undesirable plants can impair the initial development of yerba mate, which is potentiated by the lack of P. The elemental composition of the leaves can also be changed with the use of glyphosate, the addition of which can result in an increase in certain elements, including Cd and

Pb. The effects observed on growth and elemental composition differed between yerba mate clones and the type of soil; (iv) evaluating the capacity for Mn accumulation in yerba mate leaves subjected to increasing doses of Mn in two types of soil with and without liming (pot experiment). Yerba mate proved to be an Mn hyperaccumulating plant, reaching a concentration of 13.452 mg kg^{-1} without impairing plant growth. There was a difference in the leaf content of Mn according to the parent material of the soil (basalt>sandstone); and with the correction of soil acidity (without liming>with liming).

Keywords: Elemental composition. Legislation. Cadmium. Lead. Manganese.

SUMMARY

1 GENERAL INTRODUCTION 15

1.1 REFERENCES 18

2 CAPÍTULO I: CADMIUM AND LEAD CONCENTRATIONS IN YERBA MATE LEAVES FROM AGROFORESTRY AND PLANTATION SYSTEMS: AN INTERNATIONAL SURVEY IN SOUTH AMERICA 20

2.1 RESUMO 20

2.2 ABSTRACT 20

2.3 INTRODUCTION 21

2.4 MATERIALS AND METHODS 23

2.4.1 SAMPLING AND SAMPLE PREPARATION 23

2.4.2 SAMPLE DIGESTION AND ELEMENT QUANTIFICATION 23

2.4.3 QUALITY CONTROL 24

2.4.4 DETERMINATION OF CHEMICAL AND PHYSICAL SOIL PARAMETERS .24

2.4.5 CALCULATION OF INTAKE VIA INFUSION 25

2.4.6 STATISTICAL ANALYSIS 25

2.5 RESULTS 27

2.5.1 Cd AND Pb CONCENTRATIONS IN SOILS 27

2.5.2 Cd AND Pb CONCENTRATIONS IN YERBA MATE LEAVES 29

2.5.3 INTERACTION OF Cd AND Pb IN YERBA MATE LEAVES ACCORDING TO MANAGEMENT, SOIL PROPRIETIES AND PLANT NUTRITION 29

2.5.4 HEALTH RISK ASSESSMENT 36

2.6 DISCUSSION 37

2.6.1 SOIL CONTRIBUTION 37

2.6.2 MANAGEMENT AND CULTIVATION SYSTEM 39

2.6.3 HEALTH RISK ASSESSMENT 40

2.6.4 Cd AND Pb CONCENTRATION IN YERBA MATE LEAVES AND LEGISLATION 40

2.7 CONCLUSION 42

2.8 REFERENCES 43

2.9 SUPPLEMENTARY MATERIAL 50

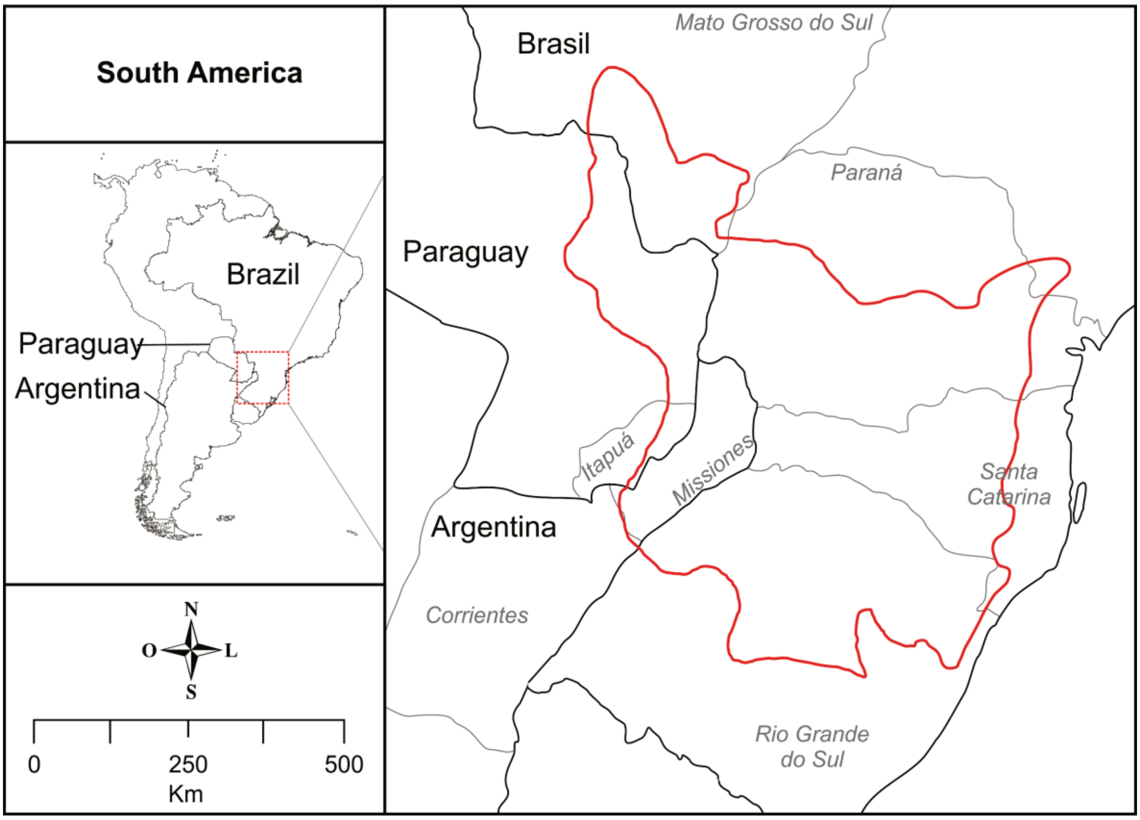
3 CHAPTER II: LINKING EDAFOCLIMATIC CONDITIONS TO THE ELEMENTARY COMPOSITION OF THE YERBA MATE LEAVES IN SOUTH AMERICA..........57
3.1 RESUMO..........57
3.2 ABSTRACT..........57
3.3 INTRODUCTION..........58
3.4 MATERIALS AND METHODS..........59
3.4.1 STUDY AREA AND SAMPLING..........59
3.4.2 ENVIRONMENTAL PARAMETERS..........60
3.5 RESULTS..........63
3.5.1 GROWING CONDITIONS AT YERBA MATE FARMS..........63
3.5.2 ELEMENTAL COMPOSITION AT YERBA MATE LEAVES..........65
3.5.3 RELATIONSHIP AMONG ELEMENTAL CONCENTRATION IN YERBA MATE LEAVES, CLIMATE, AND SOIL..........68
3.6 DISCUSSION..........71
3.7 CONCLUSION..........73
3.8 REFERENCES..........75
3.9 SUPPLEMENTAY MATERIAL..........80
4 CHAPTER III: GLYPHOSATE EFFECTS ON NON-TARGET PLANTS: COLLATERAL IMPACTS ON ELEMENTAL COMPOSITION AND GROWTH OF YERBA MATE..........83
4.1 RESUMO..........83
4.2 ABSTRACT..........83
4.3 INTRODUCTION..........84
4.4 MATERIALS AND METHODOS..........86
4.4.1 EXPERIMENTAL DESIGN..........86
4.4.2 SOIL AND PLANT ANALYSIS..........87
4.4.3 STATISTICAL ANALYSIS..........89
4.5 RESULTS..........90
4.5.1 PLANT GROWTH ATTRIBUTES..........90
4.5.2 GLYPHOSATE AND PHOSPHORUS EFFECT ON ELEMENTARY COMPOSITION..........91
4.5.3 SOIL AND GENETIC EFFECTS ON ELEMENTAL COMPOSITION..........94
4.6 DISCUSSION..........95

4.7 CONCLUSION 97
4.8 REFERENCES 98
4.9 SUPPLEMENTARY MATERIAL 104
5 CAPÍTULO IV: MANGANESE HYPERACCUMULATION CAPACITY OF *ILEX PARAGUARIENSIS* A. ST. HIL. AND OCCURRENCE OF INTERVEINAL CHLOROSIS INDUCED BY TRANSIENT TOXICITY* 106
5.1 RESUMO 106
5.2 ABSTRACT 106
5.3 INTRODUCTION 107
5.4 MATERIALS AND METHODS 109
5.4.1 EXPERIMENTAL DESIGN AND SOIL COLLECTION 109
5.4.2 DETERMINATION OF SOIL CHEMICAL AND PHYSICAL PARAMETERS 109
5.4.3 CORRECTION OF ACIDITY AND Mn DOSES 110
5.4.4 PLANT MONITORING AND ELEMENTARY QUANTIFICATION 111
5.4.5 MICROSCOPY ANALYSIS 111
5.5 RESULTS 113
5.5.1 LEAF Mn CONCENTRATION AND TOXICITY 113
5.5.2 PLANT GROWTH AND ELEMENTAL COMPOSITION 115
5.5.3 DISTRIBUTION OF ELEMENTS IN YERBA MATE LEAVES 116
5.6 DISCUSSION 117
5.6.1 ACCUMULATION OF Mn IN LEAVES 117
5.6.2 PLANT GROWTH AND ELEMENTAL INTERACTION 119
5.7 CONCLUSION 121
5.8 REFERENCES 122
5.9 SUPPLEMENTARY MATERIAL 127
6 GENERAL CONCLUSION 128
REFERENCES 129

1 GENERAL INTRODUCTION

Yerba mate (*Ilex paraguariensis* A. St-Hil), a native of South America, is one of a species of perennial trees which occur naturally in an area of approximately 540,000 km^2 in Brazil, Argentina and Paraguay (Reitz et al., 1988; Gerhardt, 2013) (FIGURE 1). According to Linhares (1969), when the Spaniards arrived in Brazil, the consumption of *chimarrão* – or *mate*, a drink made by the infusion of leaves and small branches of yerba mate, was already widespread among the indigenous people. In the middle of the 16th century, indigenous people who inhabited the basin of the Uruguay, Iguaçu and Prata rivers collected branches and leaves of yerba mate in the native forests and sold them to the populations that inhabited Chile, Bolivia and Peru. Initially, the Spanish Jesuits repudiated the consumption of yerba mate, considering *chimarrão* as being a hallucinogenic drink. However, realizing the economic potential of such a beverage, they started to popularize its consumption, thereby stimulating their trade network (Linhares, 1969).

FIGURE 1 – MAINLY NATURAL OCCURRENCE AREA OF YERBA-MATE (Red line – according to Oliveira and Rotta, 1985) IN REGIONS OF BRAZIL, ARGENTINA AND PARAGUAY.



SOURCE: Adapted from Oliveira and Rotta, 1985.

In addition to the use of yerba mate as *chimarrão*, its consumption is common in the form of *tererê* (cold infusion) and in the form of mate tea. However, with the evolution of research, several nutraceutical properties have been identified in the leaves of yerba mate (Valduga et al., 2019), and this has promoted the expansion of its use beyond traditional drinks. It is currently used as the raw material for non-traditional products such as candies, stimulating drinks, beers, flours, and various drugs (Vieira et al., 2008). With this expansion in trade, the processed yerba mate is destined for many countries around the world, with the main consumer market being South America.

In terms of legislation, the technical regulation of the Southern Common Market (MERCOSUR) defines the maximum limits for inorganic contaminants in foods marketed within South America. In this regulation, the maximum limit for Cd is 0.40 mg kg^{-1} and for Pb, 0.60 mg kg^{-1} as stipulated in the category "Teas, yerba mate, and other vegetables for infusion" (Brasil, 2013). These elements are routinely controlled by food laws due to the health risks attributed to their consumption (Hamid et al., 2019; Boskabady et al., 2018). However, after the introduction of this legislation, non-conformities in Brazilian exports started to occur, primarily due to the content of Cd and Pb being above the values established in the current legislation. As a result, questions have been raised as to the presence of these potentially toxic elements in the leaves of yerba mate. The possible existence of such contamination has been attributed to certain practices adopted in the management of the herbs. The main questions relate to the use of glyphosate and phosphate fertilizers.

In addition to the problems relating to the presence of Cd and Pb, scientific studies show a significant variation in the concentration of elements present in the leaves of yerba mate, especially metallic micronutrients and Al (Reissmann et al., 1999; Heinrichs and Malavolta, 2001; Oliva et al., 2014; Barbosa et al., 2015; Barbosa et al., 2020). However, existing studies are limited to specific regions, or refer to plants grown in greenhouses, with controlled environmental conditions. Hence, there is a need for global reassessment of the elemental composition of yerba mate leaves, seeking interactions with its origin, with the cultivation system adopted, and with the edaphoclimatic conditions of each region.

Regarding the nutritional composition of the leaves of yerba mate, the Mn content comes under the most scrutiny. Numerous studies have investigated its levels, as they frequently present values above 1,000 mg kg^{-1} (Oliva et al., 2014;

Barbosa et al., 2018; Barbosa et al., 2020). It is known that the presence of Mn is associated with soil pH (Santin et al., 2013; Toppel et al, 2018) and with the soil parent material (Motta et al., 2020). However, little is known as to the real capacity of yerba mate leaves for Mn accumulation, and even less as to the damage excessive quantities may do to plant growth.

The elementary composition of yerba mate leaves is linked to the quality of the products generated from them. Therefore, an understanding of their chemistry and composition is essential, especially as to the interactions that exist between the absorption and accumulation of these various elements. This thesis sought to explain certain scientific gaps that exist relative to the elemental composition of these leaves, specifically as to the presence of such potentially toxic elements as Cd and Pb. Current legislation relative to toxin levels was investigated as well as the multi-element composition of the yerba mate leaves and their capacity for Mn accumulation. The contribution of such variables as soil, genetics, origin and plant management were also analyzed.

1.1 REFERENCES

Barbosa, J. Z., Motta, A. C., Consalter, R., Poggere, G. C., Santin, D., & Wendling, I. (2018). Plant growth, nutrients and potentially toxic elements in leaves of yerba mate clones in response to phosphorus in acid soils. *Anais da Academia Brasileira de Ciências*, 90(1), 557-571.

Barbosa, J. Z., Motta, A. C. V., dos Reis, A. R., Corrêa, R. S., & Prior, S. A. (2020). Spatial distribution of structural elements in leaves of *Ilex paraguariensis*: physiological and ecological implications. *Trees*, 34(1), 101-110.

Barbosa, J. Z., Zambon, L. M., Motta, A. C. V. & Wendling, I. (2015). Composition, hot-water solubility of elements and nutritional value of fruits and leaves of yerba-mate. *Ciência e Agrotecnologia,* 39(6), 593–603.

Boskabady, M., Marefati, N., Farkhondeh, T., Shakeri, F., Farshbaf, A., & Boskabady, M. H. (2018). The effect of environmental lead exposure on human health and the contribution of inflammatory mechanisms, a review*. Environment international*, 120, 404-420.

Brasil. (2013). Dispõe sobre o Regulamento Técnico MERCOSUL sobre Limites Máximos de Contaminantes Inorgânicos em Alimento (Resolução-RDC nº 42, de 29 de agosto de 2013). *Diário Oficial [da] República Federativa do Brasil.*

Gerhardt, M. (2013). História ambiental da erva-mate. Tese (Doutorado em História) – Universidade Federal de Santa Catarina, Florianópolis, 290 f.

Hamid, Y., Tang, L., Sohail, M. I., Cao, X., Hussain, B., Aziz, M. Z., Usman, M., He, Z. & Yang, X. (2019). An explanation of soil amendments to reduce cadmium phytoavailability and transfer to food chain. Science of The Total Environment, 660, 80-96. https:// doi.org/10.1016/j.scitotenv.2018.12.419.

Heinrichs, R. & Malavolta, E., 2001. Composição mineral do produto comercial da erva-mate (*Ilex paraguariensis* St. Hil.). *Ciência Rural*, 31(5), 781-785.

Linhares T. (1969). História econômica do mate. Livraria J. Olympio.

Motta, A. C. V., Barbosa, J. Z., Magri, E., Pedreira, G. Q., Santin, D., Prior, S. A., Consalter, R., Young, S. D., Broadley, M. R. & Benedetti, E. L. (2020). Elemental composition of yerba mate (*Ilex paraguariensis* A. St.-Hil.) under low input systems of southern Brazil. *Science of the Total Environment,* 736, 139637.

Oliva, E. V., Reissmann, C. B., Gaiad, S., Oliveira, E. B. & Sturion, J. A., 2014. Composição nutricional de procedências e progênies de erva-mate (*Ilex* *paraguariensis* St. Hil.) cultivadas em latossolo vermelho distrófico. *Ciência Florestal*, 24(4), 793–805.

Oliveira, Y. M. M., & Rotta, E. (1985). Área de distribuição natural de erva-mate (*Ilex paraguariensis* St. Hil.). In: S*eminário sobre atualidades e perspectivas florestais*, 10., 1983, Curitiba. Silvicultura da erva-mate (*Ilex paraguariensis*): anais... Curitiba: EMBRAPA-CNPF, 1985. p. 17-36.

Reissmann, C. B., Radomski, M. I. & Quadros, R. M. B. (1999). Chemical composition of *Ilex paraguariensis* St. Hil. under different management conditions in seven localities of Paraná State. *Brazilian Archives of Biology and Technology,* 42(2), 187–194.

Reitz, R., Klein, R. M., & Rus, A. (1988). *Projeto madeira do Rio Grande do Sul: termo de referencia: estudo da materia prima: levantamento das especies florestais nativas com possibilidade de incremento e desenvolvimento*. Sudesul.

Santin, D., Benedetti, E. L., Kaseker, J. F., Bastos, M. C., Reissmann, C. B., Wendling, I. & Barros, N. F. D. (2013). Nutrição e crescimento da erva-mate submetida à calagem. *Ciência Florestal*, 23(1), 55-66.

Toppel, F. V., Junior, A. M., Motta, A. C. V., Frigo, C., Magri, E. & Barbosa, J. Z. (2018). Soil chemical attributes and their influence on elemental composition of yerba mate leaves. *Floresta*, 48(3), 425-434.

Valduga, A. T., Gonçalves, I. L., Magri, E. & Finzer, J. R. D. (2019). Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. *Food Research International*. Int. 120, 478–503.

Vieira, M. A., Rovaris, A. A., Maraschin, M., De Simas, K. N., Pagliosa, C. M., Podestá, R., Amboni, R. D. M. C, Barreto, P. L. M. & Amante, E. R. (2008). Chemical characterization of candy made of erva-mate (*Ilex paraguariensis* A. St. Hil.) residue. *Journal of agricultural and food chemistry*, 56(12), 4637-4642.

2 CHAPTER I: CADMIUM AND LEAD CONCENTRATIONS IN YERBA MATE LEAVES FROM AGROFORESTRY AND PLANTATION SYSTEMS: AN INTERNATIONAL SURVEY IN SOUTH AMERICA*

2.1 RESUMO

A atual legislação para Cd e Pb tem causado preocupação na cadeia produtiva da erva-mate. Uma investigação internacional foi conduzida para identificar a relação entre a concentração de Cd e Pb nas folhas de erva-mate e as propriedades de solo, localização geográfica, manejo agrícola, e também para avaliar a exposição humana ao Cd e Pb ao beber a infusão de erva-mate. Amostras pareadas de folhas e solo foram coletadas em 115 fazendas em sistema agroflorestal e plantações no Brasil, Argentina e Paraguai. Nós encontramos correlação entre Cd disponível no solo e nas folhas de erva-mate, bem como entre Cd e Zn foliar. O manejo de cultivo adotado não reflete em diferenças na concentração de Cd e Pb entre os solos e as folhas das plantas. O Cd (0,29 ± 0,30 mg kg^{-1}) em 21% das amostras esteve acima do limite estabelecido para as folhas de erva-mate na América do Sul (0,40 mg kg^{-1}) e 38% das amostras de folhas (0,61 ± 0,40 mg kg^{-1}) estiveram acima do limiar de Pb de 0,60 mg kg^{-1}. Os níveis legais para Cd e Pb estabelecidos para as folhas de erva-mate afetam mais o comércio do que a saúde dos consumidores, pois o consumo de Cd e Pb estimado a partir da infusão de erva-mate foi baixo.

Palavras-chave: Cd e Pb. Legislação. Risco à saúde. *Ilex paraguariensis*.

2.2 ABSTRACT

Current legislation for Cd and Pb has been causing concern in the yerba mate production chain. An international survey was conducted to identify the relation of Cd and Pb concentration in yerba mate leaves with soil properties, geographic location, agricultural management, and also to assess the exposure of humans to Cd and Pb when drinking yerba mate infusion. Paired yerba mate leaves and soil samples were collected on 115 farms from agroforestry and plantation systems in Brazil, Argentina, and Paraguay. We found correlations between available Cd in soil and yerba mate leaves, as well as between Cd and Zn foliar. Cultivation management adopted did not reflect differences in Cd and Pb concentrations between soils and the plant leaves. Cadmium (0.29 ± 0.30 mg kg^{-1}) in 21 % of the leaf samples were above the Cd threshold established for yerba mate leaves in South America (0.40 mg kg^{-1}) and 38 % of leaf samples (0.61 ± 0.40 mg kg^{-1}) were above the Pb threshold of 0.60 mg kg^{-1}. The legal levels of Cd and Pb established for yerba mate leaves affect rather their trade than consumer health, as Cd and Pb intake estimated from yerba mate infusion were low.

Keywords: Cd and Pb. Legislation. Health risk. *Ilex paraguariensis*.

2.3 INTRODUCTION

Ilex paraguariensis A. St. Hill. is called yerba mate in South America and it is an endemic tree species in southern Brazil, northeastern Argentina, and southeastern Paraguay (Valduga et al., 2019b). Yerba mate cultivation has not expanded far from the areas where it naturally grows and this species plays an important economic role for small farmers especially in remote regions. More than a third of yerba mate in South America is harvested under the canopy of native forest namely the agroforestry system. Despite this, plantations of yerba mate under full sun and subjected to fertilization and herbicide application have increased in the last decade (Marques, 2014; Santin et al., 2015).

Yerba mate infusions are widely consumed in South America (Marcelo et al., 2014) and follow specific legislation – RDC 42/2013 (Brasil, 2013) from the Southern Common Market (Mercosul), which establishes maximum concentrations of Cd (0.40 mg kg^{-1}) and Pb (0.60 mg kg^{-1}). However, it is not clear on what parameters the threshold concentrations were based as Cd and Pb baseline values for yerba mate are unknown. Since the approval of yerba mate legislation, many surveys on Cd and Pb concentrations have found concentration levels near and above the set threshold concentrations for these elements in both industrially processed leaves (Valduga et al., 2019a; Shmite et al., 2019; Milani et al., 2019; Santos et al., 2017) and non-processed yerba mate leaves (Frigo et al., 2020; Motta et al., 2020; Barbosa et al., 2020; Barbosa et al., 2018; Barbosa et al., 2015).

The industrial processing of yerba mate leaves has no potential for Cd and Pb contamination since the processes rely only on drying and crushing mature leaves (Valduga et al., 2019b). However, it is possible that the cultivation conditions of yerba mate affect Cd and Pb concentrations in its leaves because the yerba mate tree is grown in broad areas where the soil is from different parent materials and degrees of weathering (Kabata-Pendias, 2010; Rabel et al., 2018). The main soil properties that affect Cd and Pb mobility and their availability in soils include pH, organic matter (OM) content, cation exchange capacity (CEC), clay content and electric conductivity (EC) (Argüello et al., 2019; Zeng et al., 2011; Gaw et al., 2008). Yerba mate grows naturally in acidic soils, and its resistance to high levels of Al and Mn (Toppel et al., 2018; Magri et al., 2020) is well-defined, although little is known about the relationship between soil properties and levels of Cd and Pb in leaves.

In addition, Cd-Zn interaction during their uptake and translocation indicated Zn as a key element to investigate the presence of Cd in plant tissues (Gramlich et al., 2018; Argüello et al., 2019; Mitra, 2015). The role of soil in transferring Cd and Pb to yerba mate plants has been reported by Valduga et al. (2019a), who have found a high concentration of Cd in plants cultivated in igneous soil, and Pb in plants cultivated in sedimentary soil. Fertilizer application may also affect Cd and Pb concentrations in plant tissues (Gupta et al., 2014; Argüello et al., 2019), although some studies have argued that there is an overestimation of fertilizer influence on metal concentrations in soils and plants (Holmgren et al., 1993). Phosphate fertilizer can be a significant source of Cd and Pb (Moreno-Jiménez et al., 2016; Roberts, 2014) and its continuous use can introduce Cd and Pb into agricultural systems (Gupta et al., 2014).

Another aspect is food safety and it is important to assess the contribution of potentially toxic elements from crops and determine whether their consumption is safe (Škrbić and Čupić, 2005; Škrbić and Cvejanov, 2011). The Joint Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) has established a provisional tolerable weekly intake (PTWI) 7 μg kg^{-1} body weight of Cd and 25 μg kg^{-1} body weight of Pb (Joint FAO/WHO Expert Committee on Food Additives, 2010). The exposure of humans to Cd and Pb above these values can be harmful to health and it is, therefore, essential to assess the exposure of consumers of yerba mate infusions to Cd and Pb and evaluate potential risks.

By considering the above discussion, a large-scale survey on Cd and Pb concentrations in leaves of yerba mate plants grown in the natural area of the species was conducted, including the main producing regions in South America. The survey aimed: (i) to identify the contribution of soil type, geographic location and crop system to concentrations of Cd and Pb in soil and yerba mate leaves; (ii) to identify production sites of yerba mate leaves potentially affected by Cd and Pb; (iii) to determine baseline concentrations of Cd and Pb in yerba mate leaves grown under natural conditions and cultivation systems; and (iv) to evaluate the exposure of consumers to Cd and Pb by yerba mate infusions.

2.4 MATERIALS AND METHODS

2.4.1 SAMPLING AND SAMPLE PREPARATION

Samples of soil and yerba mate leaves were collected from farms in Brazil, Argentina, and Paraguay between December 2017 and January 2018. The selected farms are located in the main producing centres of yerba mate in South America, including 115 farms (FIGURE S1). Details of each sampled site are given in TABLE S1. The cultivation system at each farm was checked during sampling and information on parent material of soil was previously obtained from geology maps and confirmed by local observation.

Soil samples were collected from 0 to 20 cm soil layer by using a Dutch auger and left to dry at ambient temperature until constant weight. Then, the soil samples were quartered, manually ground in a porcelain mortar, and passed through a 10 mesh sieve (2 mm) for chemical and physical analyses. Soil samples were further ground and passed in a 70 mesh sieve (0.21 mm) for the quantification of pseudototal concentrations of elements.

Mature leaves of yerba mate were collected on the upper third of plant crowns, from four cardinal points to form composite samples from 20 plants. After harvesting, plant material from each farm was homogenized, dried in a forced-air circulation oven at 65 °C until constant weight, crushed in a ball mill and passed through a sieve of 30 mesh (0.25 mm).

2.4.2 SAMPLE DIGESTION AND ELEMENT QUANTIFICATION

Soil extraction for the determination of pseudototal concentrations of Fe, Mn, P, Cu, Zn, Sb, As, Cd, and Pb was performed according to USEPA 3051A method (Usepa, 1995): 400 mg of soil, 9 mL of 65 % HNO_3 (Merck) and 3 mL of 36–38 % HCl were mixed in polypropylene tubes. The mixture was submitted to microwave heating (Mars 6 – CEM corporation) at 1200− 1800 W for 5.5 min at the maximum temperature of 175 °C for 4.5 min. At the end of the extraction, the volume mixture was made up to 50 mL using purified water and filtered through a 0.45 μm filter paper. The water used for the preparation of all samples and solutions was purified (resistivity of 18.2 MΩ cm) in a Milli-Q system (Millipore Milli-Q Academic). The quantification of elements was performed by inductively coupled plasma optical emission spectrometry – ICP OES (a Varian 720-ES instrument was employed).

For yerba mate digestion, 4 mL of 65 % HNO_3 (Merck), 1 mL of H_2O_2, and 3 mL of water were added to 200 mg of dried-ground leaves and digested assisted by microwave heating at 1800 W for 20 min at the maximum temperature allowed and 180 °C for 15 min. At the end of the digestion, the digestate volume was made up to 50 mL with water. Concentrations of Cd and Pb were determined by Graphite Furnace Atomic Absorption Spectrometry GFAAS (instrument model AA 6800 from Shimadzu, Japan) and followed the heating programs shown in TABLE S2. The concentrations of other investigated elements were determined by ICP OES.

2.4.3 QUALITY CONTROL

Samples were analyzed in duplicate, mean values were calculated, and precision was based on the values of relative standard deviation (RSD). When the RSD was higher than 10% the sample solutions were discarded and the chemical analysis of these samples was repeated.

Analytical blanks and certified reference material (CRM) of soil (2710A – Montana I soil) and plant (GBW 10,052 – *Camellia sinensis*) were analyzed as for the soils and yerba mate leaves samples for accuracy evaluation. The limit of detection (LOD) was based on the ratio of 3 times the standard deviation (3s) of 10 measurements of soil blank divided by the slope of the calibration curve. The same approach was followed to estimate the limit of quantification (LOQ), but in this case, 10s was computed instead of 3s. For yerba mate leaves, the LOD and LOQ of the method were calculated by using data from a sample with a low known concentration of the analyte, with the same calculations as previously described. Quality control followed the methodology of Inmetro (2020), and respective results are shown in TABLE S4 and S5.

2.4.4 DETERMINATION OF CHEMICAL AND PHYSICAL SOIL PARAMETERS

Soil samples were analyzed for pH (1:2.5 soil-$CaCl_2$ 0.01 M solution ratio), electrical conductivity (EC) (1:5 soil-water ratio), organic matter content (Walkley Black method), cation exchange capacity (CEC) at pH 7, and concentrations of Mn, Fe, Zn, Cu, Ni, Cd, and Pb after soil extraction with Mehlich-I solution (Marques and Motta, 2003). Clay content was determined according to the Bouyoucos hydrometer method.

2.4.5 CALCULATION OF INTAKE VIA INFUSION

The weekly intake of Cd and Pb by consumption of yerba mate infusions was calculated to assess the health risk as reported by Škrbić and Čupić (2005) Škrbić and Cvejanov (2011). The estimation of metal intake from tea demands the evaluation of their solubility in water (Han et al., 2006). Different water-soluble Cd values have been observed for yerba mate leaves. Shmite et al. (2019) have reported that 7–67% of total Cd in yerba mate leaves are soluble in water; Santos et al. (2017) have found that 10 % of total Cd in leaves are water-soluble and Barbosa et al. (2015) have calculated 50–60 % of the same. As for Cd, Pb water-solubility values reported also varies widely: Santos et al. (2017) have found approximately 5% of the total Pb in yerba mate infusion; Shmite et al. (2019) have found up to 35 % of the total Pb in infusions and Barbosa et al. (2015) about 35–60 %. Due to these variations, an average solubility of 50 % can be assumed for calculating the weekly intake of both elements.

Thus, to calculate the average weekly ingestion (μg kg^{-1} body weight), the values found in this study were multiplied by 0.5 (watersoluble portion) and by the average daily consumption. Daily consumption was based on annual average consumption per capita (kg): Uruguay = 8.6; Argentina = 6.5; Paraguay = 2.5; Brazil = 0.8 (Mendoza, 2020). The average body weight of 60 kg was adopted for the same region.

2.4.6 STATISTICAL ANALYSIS

The uptake factor (UF) was calculated by dividing the element concentrations in yerba mate leaves by the pseudototal concentration of the same elements in soil (Alexander et al., 2006). Pseudototal and available Cd and Pb concentrations in soils were discriminated according to their parent materials by using Kruskal Wallis (Bewick et al., 2004) test followed by Dunn's Test ($p < 0.05$). For each parent material, the pseudototal and available concentrations of Cd and Pb were discriminated according to agronomic factors and cultivation system (plantation versus agroforest, fertilizer and/or herbicide use and sampling region) by Wilcoxon test ($p < 0.05$) (Whitley and Ball, 2002).

Principal component analysis (PCA) included available and pseudototal concentrations of all the quantified elements in the soil. Prior to PCA, data were checked for normality by Shapiro-Wilk test. Data were transformed by Box-Cox when necessary, ensuring normal data distribution. PCA was performed from the correlation matrix of standardized data for mean zero and variance one. The number of Principal Components (PC) was determined by Kaiser's rule (Kaiser and Rice, 1974). The interpretation of the results of the PCA followed the recommendations of Stafilov et al. (2011).

Spearman correlations were applied among soil proprieties. Cadmium and Pb concentration in yerba mate leaves and UF of these elements were investigated. Cadmium and Pb contents in yerba mate leaves were compared according to the agronomic factors (Cultivation system – plantation versus agroforest and fertilizer or herbicide use) and sampled region by Wilcoxon test ($p < 0.05$). Multivariate and univariate regression analyses were conducted to select soil variables that would predict Cd and Pb levels in yerba mate leaves. Regression trees of concentrations of Cd and Pb in yerba mate leaves as a function of all soil and plant variables were performed by using the function ctree of "party" package (Hothorn et al., 2006). Statistical analysis was performed using R software version 3.6.0 (R Core Team, 2019).

2.5 RESULTS

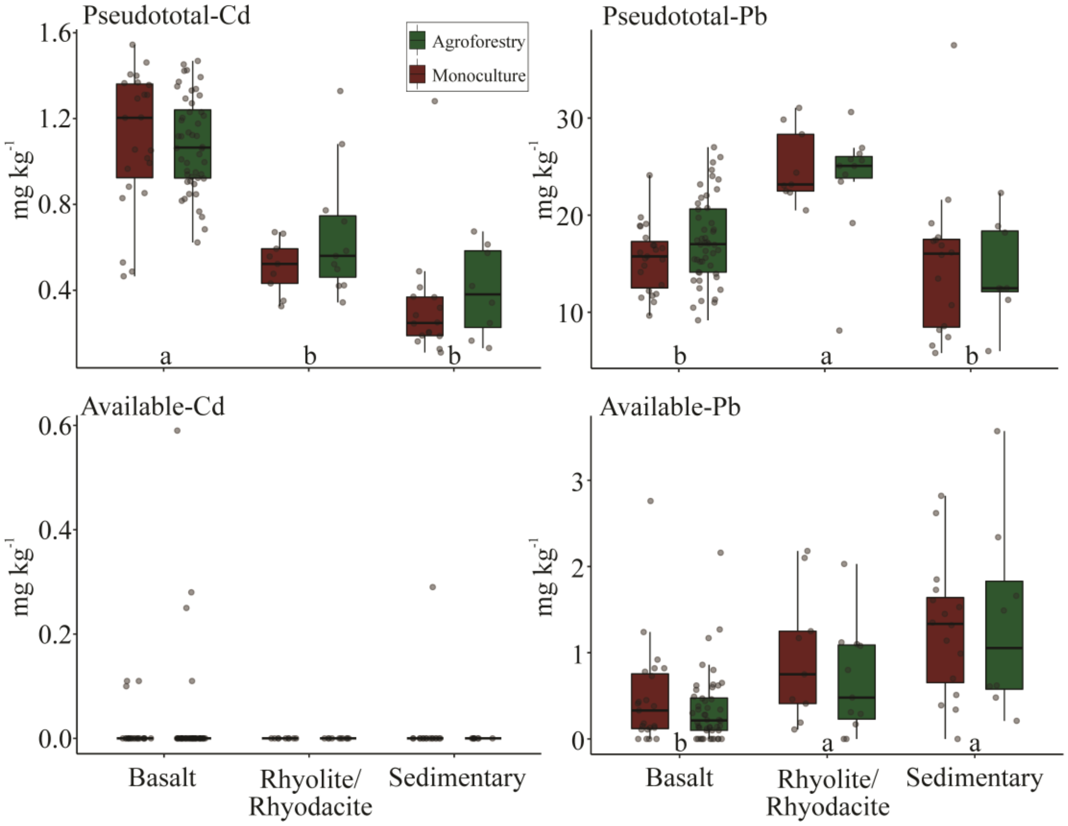
2.5.1 Cd AND Pb CONCENTRATIONS IN SOILS

Pseudototal concentrations of Cd and Pb in soils ranged from 0.11 to 1.54 mg kg^{-1} and 5.80–37.51 mg kg^{-1}, respectively. Soil parent material properties affect Cd and Pb pseudototal concentrations in soil ($p < 0.01$). Pseudototal concentration of Cd was higher in basaltic soil than in other soil types, while that of Pb was higher in rhyolite/rhyodacite soils (FIGURE 1). Yerba mate cultivation system and fertilizer application did not affect Cd and Pb pseudototal concentration in soil ($p > 0.05$).

Available Cd in soils was detected only in 8 samples out of 115 (7%) and concentrations ranged from 0.10 to 0.59 mg kg^{-1} (FIGURE 1). Seven of these 8 samples were basaltic soil (5 samples from Brazil and 3 from Paraguay). Available Pb was detected in 97 samples (84%) and the element concentration ranged from 0.10 and 3.57 mg kg^{-1}. The concentration of available Pb was lower in soil from basalt than in soils from other parent materials (FIGURE 1). Available Pb was not detected in 18 soil samples, which 17 of these samples of basaltic soil and 1 sample of sedimentary soil. The concentrations of available Cd and Pb were similar for the two evaluated cultivation systems and fertilized and nonfertilized sites within the same soil type ($p > 0.05$).

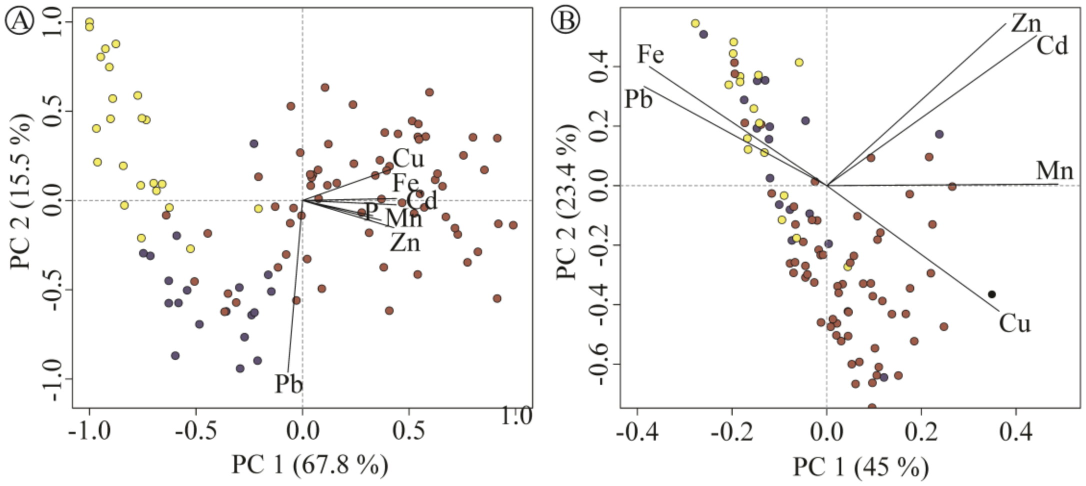
Principal Component Analysis (PCA) showed a significant relationship between Cd and Pb and other investigated elements (FIGURE 2A and FIGURE 2B; TABLE S5). Pseudototal element concentrations explained 71% of data variance and indicated that Cd, P, Cu, Fe, Mn, and Zn concentrations were correlated in soils. These elements are mostly associated with basalt parent material. There were positive correlations between available Cd and Zn, as well as between available Pb and Fe. A negative correlation between available Pb and Cu was observed (FIGURE 2B and TABLE S5).

FIGURE 1 – PSEUDOTOTAL AND AVAILABLE Cd AND Pb CONCENTRATIONS IN SOILS ACCORDING TO PARENT MATERIAL. VALUES WITH THE SAME LOWERCASE LETTERS DO NOT STATISTICALLY DIFFER BY KRUSKAL-WALLIS TEST ($p < 0.05$) FOLLOWED BY DUNN TEST.



SOURCE: The author (2021).

FIGURE 2 – PRINCIPAL COMPONENTS (PC-1 AND PC-2) FOR (A) PSEUDOTOTAL CONCENTRATIONS OF Fe, Mn, P, Cu, Zn, Cd, AND Pb iN SOIL (N = 115), (B) AVAILABLE CONCENTRATIONS OF Fe, Mn, Cu, Zn, Cd, AND Pb IN SOILS (N = 115) COLLECTED FROM THE TOPSOIL (0–20 CM).



SOURCE: The author (2021).

LEGEND: Red, blue, and yellow points refer to basalt soil, rhyolite/rhyodacite soil and sedimentary soil, respectively.

2.5.2 Cd AND Pb CONCENTRATIONS IN YERBA MATE LEAVES

The overall mean concentrations of Cd and Pb in yerba mate leaves were 0.29 mg kg^{-1} and 0.61 mg kg^{-1}, respectively. But Cd concentration in yerba mate leaves ranged from 0.10 to 1.61 mg kg^{-1}, and that of Pb ranged from 0.11 to 2.59 mg kg^{-1}. FIGURE S2 shows the distribution of concentration frequencies for Cd and Pb in yerba mate leaves and FIGURE 3 shows each concentration value according to the sample location. These frequencies showed that yerba mate leaves collected in 24 farms (21%) had Cd concentration above the legal limit of 0.40 mg kg^{-1}. The same analysis for Pb levels revealed that 44 samples (38%) had Pb concentration above the legal limit of 0.60 mg kg^{-1} (Brasil, 2013). In 10 leaf samples (9%) both elements were present at concentrations higher than the established limit. In other words, 52% of the samples showed non-compliance with Cd and/or Pb considering the current legislation.

2.5.3 INTERACTION OF Cd AND Pb IN YERBA MATE LEAVES ACCORDING TO MANAGEMENT, SOIL PROPRIETIES AND PLANT NUTRITION

The measured soil properties showed a large variability (TABLE 1) and weak correlations (Spearman coefficient) with Cd and Pb concentrations in yerba mate leaves and uptake factor (FIGURE 4). More than half of the analyzed soil samples had pH ($CaCl_2$) below 4, approximately 44% between 4 and 5, and only 6% of samples had pH above 5, revealing the predominance of acid soils. A reasonable positive correlation was observed between soil pH and soil pseudototal-Cd concentration ($r = 0.44$; $p < 0.01$), and negative correlation between soil pH and soil available-Pb concentration ($r = 0.46$; $p < 0.01$). Cadmium in yerba mate leaves positively correlated with soil pseudototal-Cd ($r = 0.35$; $p < 0.01$) and soil available-Cd ($r = 0.49$; $p < 0.01$). The same analysis for Pb (yerba mate leaves versus soil) revealed insignificant correlation for pseudototal and available forms of this element ($p > 0.05$).

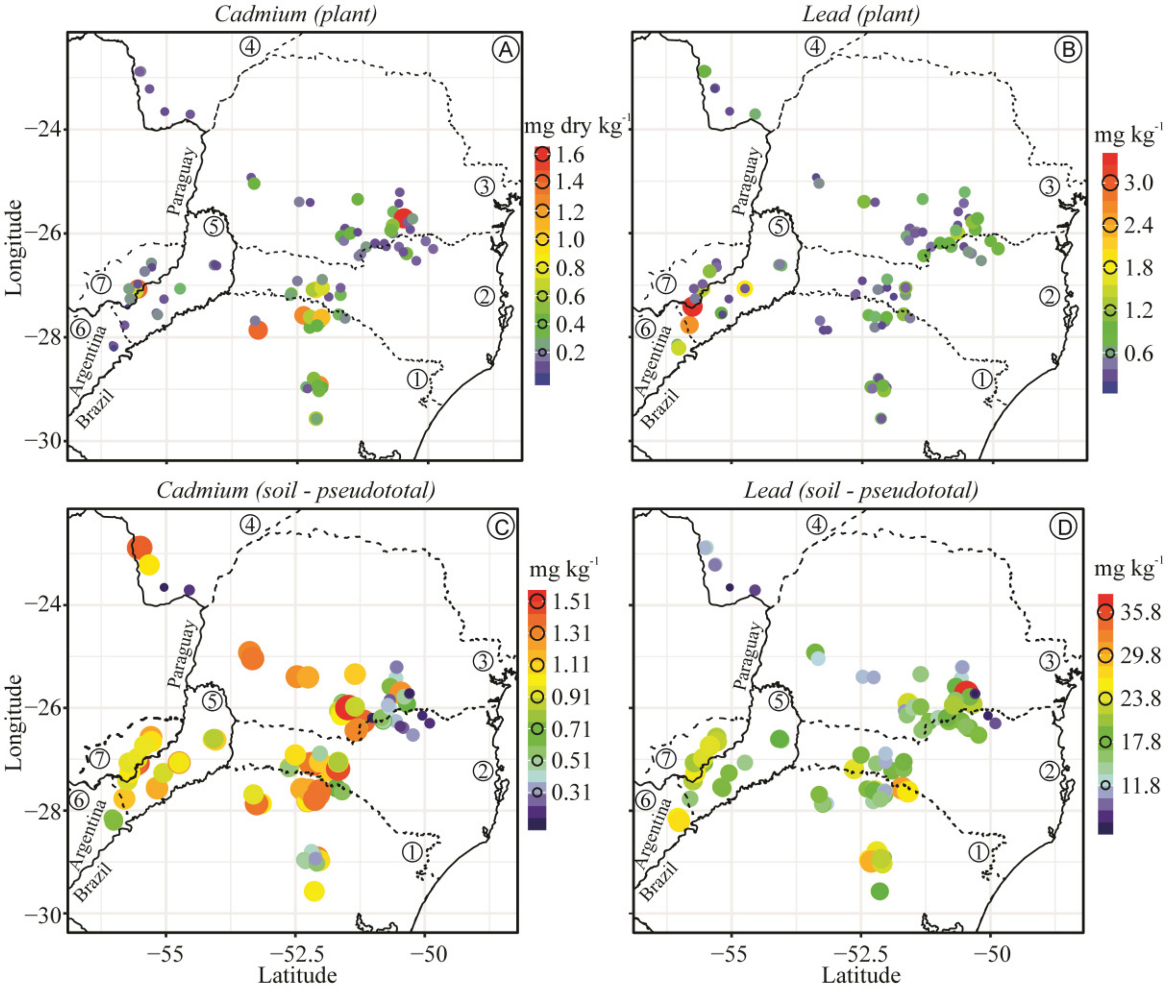
TABLE 1 – MEASURED PROPRIETIES AND UPTAKE FACTOR OF Cd AND Pb FOR 115 SOIL SAMPLES FROM THE MAIN YERBA MATE PRODUCING SITES IN SOUTH AMERICA.

Proprieties[a]	Range	Mean
pH	3.43–6.71	4.17
CEC ($\text{cmol}_c\ \text{dm}^{-3}$)	4.88–30.42	16.45
OM (%)	1.17–14.53	6.68
EC ($\mu\text{S cm}^{-1}$)	71.6–810.0	235.9
Clay (%)	12.5–83.7	60.2
Zn-pseudototal (mg kg^{-1})	2.56–185.58	71.77
Mn-pseudototal (mg kg^{-1})	34.98–4652.73	869.65
Zn-available (mg kg^{-1})	0.50–52.00	5.93
Mn-available (mg kg^{-1})	2.0–645.0	107.8
Cd UF	0.01–2.12	0.43
Pb UF	0.006–0.168	0.040

SOURCE: The author (2021).

LEGEND: a CEC: cation exchange capacity at pH 7, OM: Organic matter, EC: electrical conductivity, UF: uptake factor.

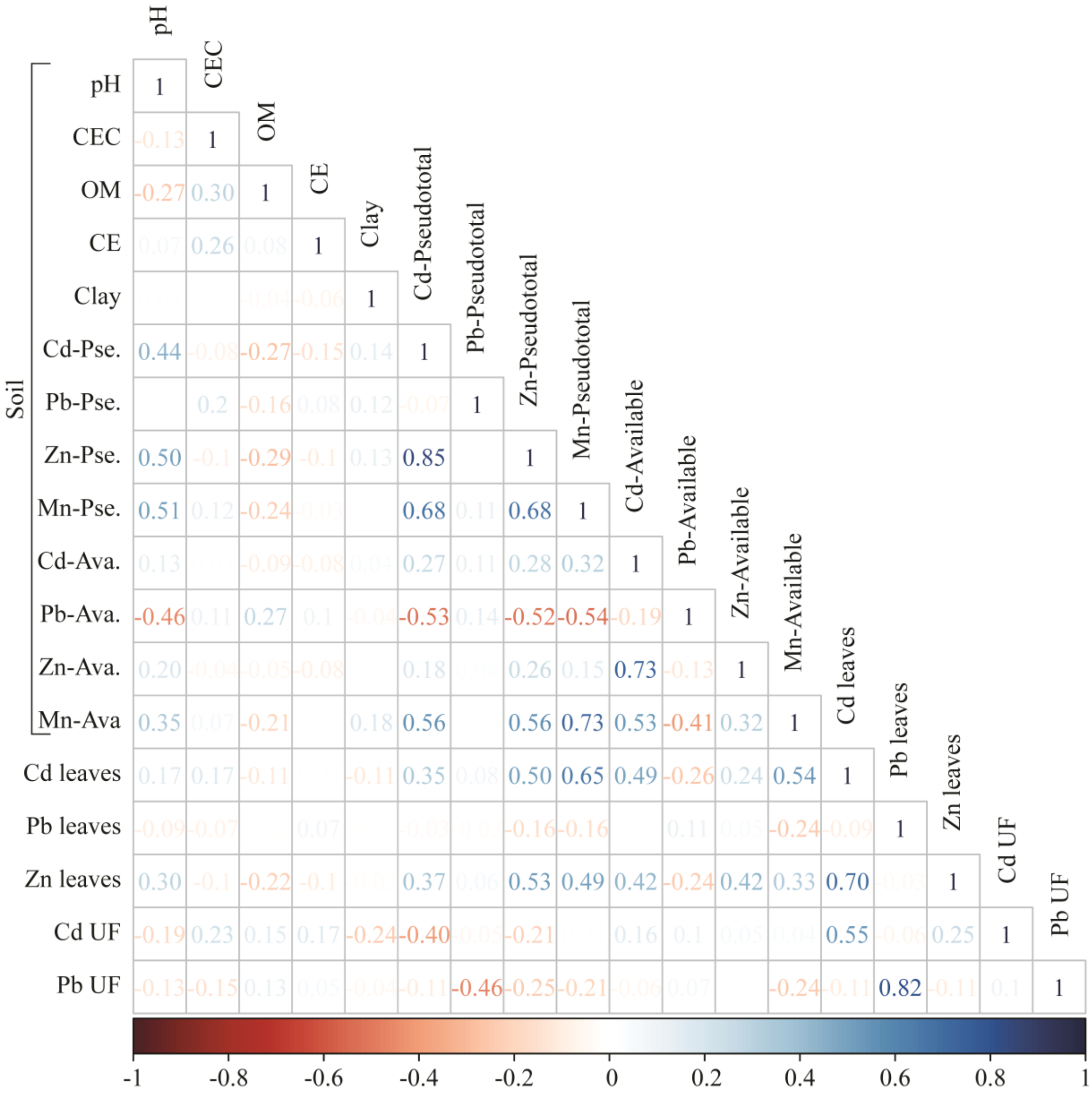
FIGURE 3 – CONCENTRATIONS OF Cd (A) AND Pb (B) IN YERBA MATE LEAVES AND PSEUDOTOTAL CONCENTRATIONS OF Cd (C) AND Pb (D) IN SOIL SAMPLES. SOLID LINES DEFINE THE BORDERLINES BETWEEN COUNTRIES AND DASHED LINES REPRESENT THE LIMITS BETWEEN STATES, PROVINCES, AND DEPARTMENTS WITHIN COUNTRIES.



SOURCE: The author (2021).

LEGEND: (1) *Rio Grande do Sul*-BR; (2) *Santa Catarina*-BR; (3) *Paraná*-BR; (4) *Mato Grosso do Sul*-BR; (5) *Missiones*-AR; (6) *Corrientes*-AR; (7) *Itapuá*-PY.

FIGURE 4 – CORRELOGRAM (SPEARMAN CORRELATION) BETWEEN SELECTED PROPRIETIES OF SOILS, Cd AND Pb IN YERBA MATE LEAVES AND UPTAKE FACTOR OF Cd AND Pb.



SOURCE: The author (2021).

LEGEND: CEC= Cation exchange capacity at pH 7; OM= Organic matter, EC: Electrical conductivity, PSE: Pseudototal, AVA: Available, UF: Uptake factor.

Levels of Cd in yerba mate leaves were not affected by cultivation system (agroforestry and plantation), chemical fertilizer, herbicide, and soil parent material ($p > 0.05$ for both) (FIGURE 5A-D). However, there were significant differences between locations ($p < 0.05$), with higher Cd concentration values in yerba mate from Rio Grande do Sul-BR and Paraguay (TABLE 2 and FIGURE 3A). Lead concentration in yerba mate leaves was slightly higher for areas where fertilizers were used ($p = 0.02$) (FIGURE 5E-H).

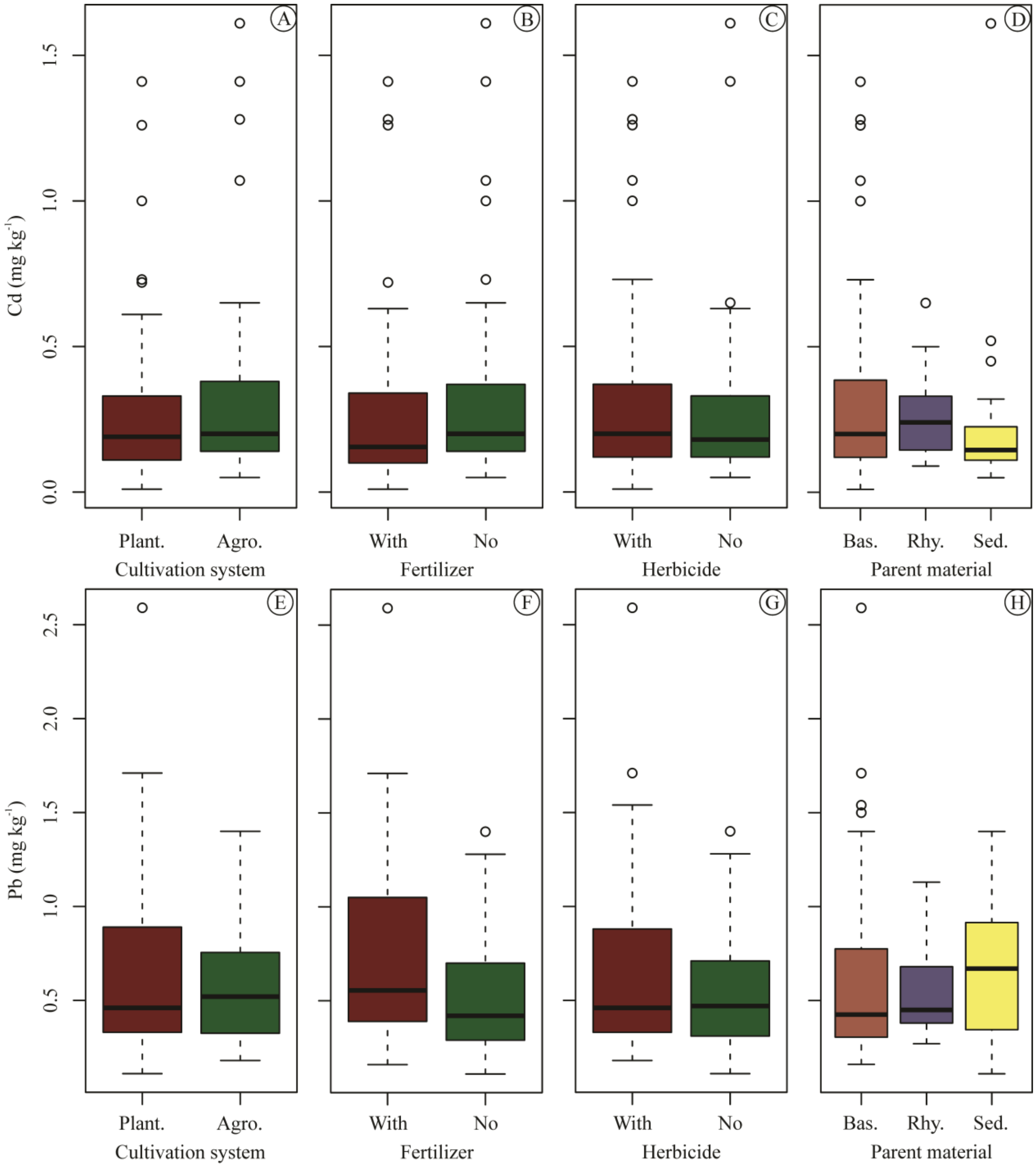
TABLE 2 – Cd AND Pb CONCENTRATIONS (MEAN ± STANDARD DERIVATION) IN YERBA MATE LEAVES ACCORDING TO SAMPLING LOCATION AND RESULT OF THE KRUSKAL-WALLIS TEST FOLLOWED BY DUNN'S TEST (p <0.05).

Region	**n**	**Cd (mg kg^{-1})**	**Pb (mg kg^{-1})$^{n.s.}$**
Brazil			
Rio Grande do Sul	35	0.41±0.35^{a}	0.47±0.25
Santa Catarina	21	0.27±0.24^{ab}	0.53±0.27
Paraná	31	0.26±0.28^{ab}	0.66±0.37
Mato Grosso do Sul	6	0.12±0.05^{b}	0.66±0.54
Argentina	12	0.13±0.08^{b}	0.90±0.70
Paraguay	10	0.38±0.43^{ab}	0.72±0.49

SOURCE: The author (2021).

LEGEND: $^{n.s.}$, non-significantly. Different letters in the same column represent statistical difference by Kruskal-Wallis test followed by Dunn's test (p <0.05)

FIGURE 5 – Cd AND Pb CONCENTRATIONS IN YERBA MATE LEAVES ACCORDING TO CULTIVATION SYSTEM, FERTILIZER AND HERBICIDE USE, AND SOIL TYPE. AGRO.= AGROFOREST SYSTEM; PLANT.= PLANTATION. BAS.= BASALT; RHY.= RHYOLITE/RHYODACITE; SED.= SEDIMENTARY. STATISTICAL DIFFERENCE WAS OBSERVED ONLY FOR PB IN RELATION TO THE USE OF FERTILIZERS (WILCOXON TEST – $p < 0.05$).



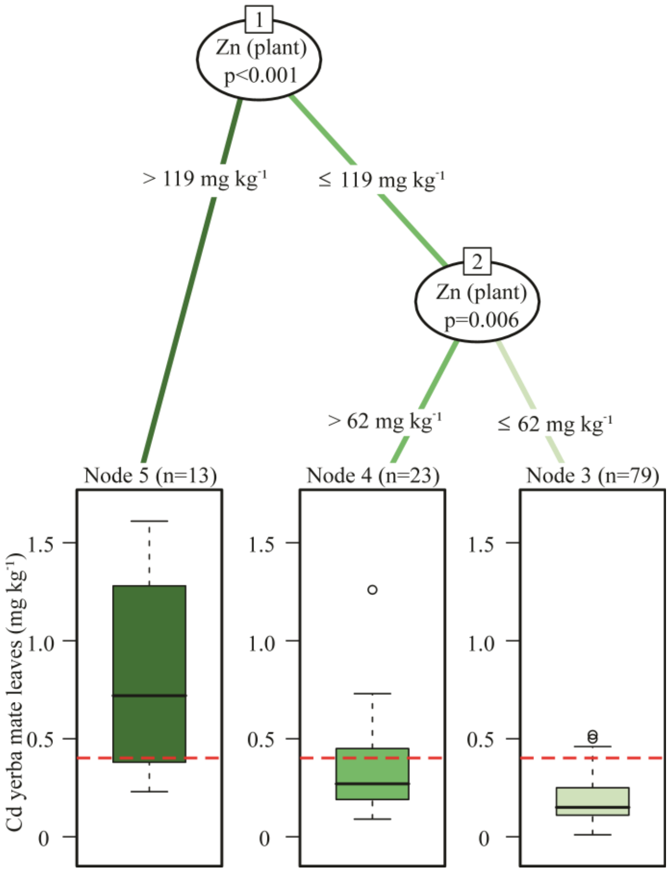
SOURCE: The author (2021).

Two multivariate regression models were generated considering Cd in yerba mate leaves as the response variable. The first model showed a relationship among Cd in yerba mate leaves and the pseudototal concentrations of Mn, Zn and Cd ([Cd-leaf] = 0.121 + 0.0003 x [Mn] + 0.004 x [Zn] - 0.392 x [Cd]; R2 = 0.50; p < 0.01). The second model revealed a similar correlation among available levels of Cd and Mn ([Cd-leaf] = 0.163 + 0.001 x [Mn] + 1.137 x [Cd]; R2 = 0.56; p < 0.01).

Cadmium and Zn concentrations in yerba mate leaves showed a strong correlation (r = 0.70; p < 0.01) which was confirmed by the regression tree (FIGURE 6) with the formation of three distinct groups: leaves with more than 119 mg kg^{-1} of Zn also had higher Cd concentration, followed by those with Zn concentration between 119 and 62 mg kg^{-1}. The last group of samples had Zn concentration lower than 62 mg kg^{-1} and the lowest Cd concentration.

No correlation between chemical and physical proprieties of soil and plant nutrition with respect to Pb concentration in yerba mate leaves were observed. Additionally, it was observed that the Pb concentration was higher than that allowed by the current legislation for all sampled regions (TABLE 2 and FIGURE 3B).

FIGURE 6 – REGRESSION TREES REFERRING TO CD CONCENTRATION IN YERBA MATE LEAVES AS A FUNCTION Zn CONCENTRATION. DASHED RED LINE REPRESENTS THE LEGAL LIMIT OF Cd FOR YERBA MATE IN SOUTH AMERICA.



SOURCE: The author (2021).

2.5.4 HEALTH RISK ASSESSMENT

The weekly Cd intake from drinking yerba mate infusion was estimated to be 12.82 µg per person, equivalent to 0.21 µg kg^{-1} body weight (60 kg per person), which represents 3.05 % of the PTWI, based on the average Cd concentration found in this work – 0.29 mg kg^{-1}. However, considering the maximum Cd concentration found in this work (1.61 mg kg^{-1}) and the highest per capita consumption of yerba mate infusion (Uruguay= 8.64 kg per capita), Cd ingestion was estimated to be 133.30 µg per person, equivalent to 2.22 µg kg^{-1} body weight, which represents 31.74 % of the PTWI (TABLE 3).

For Pb, the weekly intake from drinking yerba mate tea was estimated to be 26.97 µg per person, equivalent to 0.45 µg kg^{-1} body weight – 1.80 % of the PTWI, when using the average Pb concentration found in this work. Considering the maximum Pb concentration found in this work (2.59 mg kg^{-1}) and yerba mate consumption in Uruguay (8.64 kg per capita), Pb ingestion was estimated to be 214.74 µg per person, equivalent to 3.58 µg kg^{-1} body weight, which represents 14.32 % of the PTWI (TABLE 3).

TABLE 3 – ESTIMATED CONSUMER INTAKE OF Cd AND Pb FROM YERBA MATE TEA IN THE MAIN CONSUMING COUNTRIES OF THIS DRINK IN SOUTH AMERICA.

	Uruguay[2]	Argentina	Paraguay	Brazil	General
Annual average consumption per capita (kg)[1]	8.6	6.5	2.5	0.8	4.61
Normal scenery (Cd)					
Cd average (mg kg^{-1})	0.31	0.13	0.38	0.31	0.29
Cd solubility (%)	50	50	50	50	50
Weekly ingestion per person (µg)	25.3	8.3	9.2	2.3	12.8
Weekly ingestion (µg kg^{-1} body weight)	0.42	0.14	0.15	0.04	0.21
% of the PTWI[3]	6.0	2.0	2.2	0.6	3.1
Extreme scenery (Cd)					
Maximum of Cd (mg kg^{-1})	1.61	0.29	1.41	1.61	1.61
Weekly ingestion per person (µg)	133.30	18.19	33.70	12.34	71.13
Weekly ingestion (µg kg^{-1} body weight)	2.22	0.30	0.56	0.21	1.19
% of the PTWI	31.7	4.3	8.0	2.9	16.9
Normal scenery (Pb)					
Pb average (mg kg^{-1})	0.56	0.90	0.72	0.56	0.61
Cd solubility (%)	50	50	50	50	50
Weekly ingestion per person (µg)	46.68	55.97	17.32	4.32	26.97
Weekly ingestion (µg kg^{-1} body weight)	0.78	0.93	0.29	0.07	0.45
% of the PTWI	3.1	3.7	1.2	0.3	1.8
Extreme scenery (Pb)					
Maximum of Pb (mg kg^{-1})	2.59	2.59	1.50	2.59	2.59
Weekly ingestion per person (µg)	214.74	161.55	35.96	19.88	114.58
Weekly ingestion (µg kg^{-1} body weight)	3.58	2.69	0.60	0.33	1.91
% of the PTWI	14.3	10.8	2.4	1.3	7.6

SOURCE: The author (2021).

LEGEND: [1]Data according Mendoza, 2020. [2]As the yerba mate sold in Uruguay comes from Brazil, the reference values for Cd and Pb in these countries are the same. [3]Joint FAO/WHO Expert Committee on Food Additives established a provisional tolerable weekly intake (PTWI) – Joint FAO/WHO Expert Committee on Food Additives (2010).

2.6 DISCUSSION

2.6.1 SOIL CONTRIBUTION

High concentrations of metals were found in soils from igneous rock and corroborated those results reported by Althaus et al. (2018), who have found higher values of Cd, Cr, Cu, Ni, V, Zn, and Pb in soils of igneous origin than in sedimentary soils. This could be the reason for the higher concentrations of Cd and Pb in soils collected for the present study and their correlations with the other investigated metals. Such correlations suggest that the largest part of Cd and Pb in soils has originated from the parent material rather than from agricultural management.

The influences of OM, CEC, EC and clay content on Cd and Pb availability and accumulation in yerba mate leaves were not observed. The inconsistency of the relationship between Cd and Pb in soil and the higher concentrations values of Cd and Pb in yerba mate leaves may be attributed to variations in environmental conditions among sampling sites. The pH was negatively correlated with available Pb suggesting that acidic soils have more available-Pb that can be reduced at high soil pH. The Pb availability decreases as a response to high pH by increasing soil adsorption and consequently decreasing Pb mobility and bioavailability (Kabata-Pendias, 2010; Martınez-Villegas et al., 2004). Although no significant correlation has been observed for yerba mate leaves in this work, extremely acidic soils can be the main factor of Cd accumulation in plants in uncontaminated soils (Wang et al., 2015). This can justify the Cd concentration values in yerba mate leaves since some soil samples with extremely low pH were found in this study. The increase of pH (about 6) was shown to be efficient for reducing the Cd uptake by plants (Yang et al., 2018). However, yerba mate does not grow well in soils at pH close to 6 (Poletto et al., 2011).

Differences in pseudototal concentrations of Cd and Pb in soils did not predict the same behaviour for yerba mate leaves, differently to what was observed for *C. sinensis* (Yaylalı-Abanuz and Tüysüz, 2009). In the present study, available Cd concentration in soil was a better predictor of Cd in plant tissue than pseudototal-Cd concentration, which has also been observed by McLaughlin et al. (2011).

Cadmium showed a positive correlation with pseudototal and available Zn in soil as well as Cd and Zn in yerba mate leaves (Fig. 2 and Fig. 4). According to Reeves et al. (2018), high Cd concentration in soil is dependent on Zn ores. These elements have similar chemical properties (Rizwan et al., 2017a) as both of them are chalcophile (Goldschmidt classification), predominantly in the form of divalent cations with similar ionic radius (Cd^{2+}= 0.103 nm; Zn^{2+}= 0.083 nm). Studies have reported dubious behaviour of Cd and Zn uptake, sometimes reporting synergistic interactions (Reeves et al., 2018; Molitor et al., 2005) or antagonistic interactions (Rizwan et al., 2017b; Troadec et al., 2010). Results obtained in the present study suggest the existence of synergistic effects between Zn and Cd in yerba mate tissue, which can be attributed to the similarity between these elements. Molitor et al. (2005) have reported a strong correlation between Cd and Zn in *Thiaspi caerulesce* and suggested that it is indicative of the existence of a common transport system of these metals. Grotz and Guerinot (2006) have identified Zn-Fe permease (ZIP), as a protein responsible for metal transport in many dicotyledonous species and have concluded they are involved in the transport of Mn^{2+}, Fe^{2+}/Fe^{3+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, and Zn^{2+}. Saifullah et al. (2016) have shown that foliar application of Zn in wheat reduced Cd concentration in leaves due to reduction of Zn and Cd plant uptake, confirming that Cd depends on Zn uptake rate.

The concentration of Pb in the soil was higher than in the yerba mate leaves and Cd uptake and accumulation in *I. paraguariensis* was lower compared to Pb. On the other hand, Cd concentration in the plant leaves was very close or sometimes higher than in soil. Such a difference may be associated with a lower rate of Pb translocation since trees have a high capacity to accumulate Pb in roots and precipitate this element in the rhizosphere, which reduces their translocation to aerial parts (Kidd et al., 2015).

2.6.2 MANAGEMENT AND CULTIVATION SYSTEM

The concentration of Cd in yerba mate leaves was not affected by crop system (agroforestry or plantation), fertilizer and herbicide application (Fig. 5). Actually, the found concentrations of Cd represented the natural values of this metal in the species tissue (baseline level). Fertilizer application was the only factor apparently related to Pb levels in yerba mate leaves but it demands further investigation as fertilization did not change Pb concentrations in the sampled soils. Additionally, 80 % of fertilized sites of this study were in Argentina, Paraguay and Rio Grande do Sul state in Brazil where Pb concentration was higher in the soil than in non-fertilizer sites (mean of 18.91, 21.80, and 20.29 mg kg^{-1}, respectively). In this sense, Barbosa et al. (2018) have reported dubious results of Pb concentration in leaves of yerba mate plants grown in soil where P fertilizer had been applied, with a reduction in Pb concentration in yerba mate leaves for some pH conditions.

The average Pb concentration (0.54 mg kg^{-1}) in leaves of yerba mate grown in non-fertilizer soils was very close to the limit established by Mercosul legislation (Brasil, 2013), but Pb concentrations in some of these samples were more than twice this limit (maximum measured value of 1.40 mg kg^{-1}). Atmospheric particle deposition can contribute to Pb concentration in plant leaves (Ma et al., 2019), an effect that is more common near major urban centres, especially close to industrial areas (Wang et al., 2015) and in areas adjacent to heavy vehicular traffic (Werkenthin et al., 2014). Frigo et al. (2020) disregarded the contamination by Cd and Pb due to the proximity of highways, however, according to Motta et al. (2020), dust deposition can contribute to high concentrations of Pb in the leaves of yerba mate, with an increase of 20%. Yerba mate leaves are processed without any washing (Valduga et al., 2019b), which may contribute to higher Pb levels in the final product.

2.6.3 HEALTH RISK ASSESSMENT

Considering the weekly intake of Cd and Pb reported, even for the most extreme possible scenario, Cd and Pb intake by the consumption of yerba mate infusion would not be far away from the PTWI recommended by the Joint FAO/WHO Expert Committee on Food Additives (2010). Consequently, yerba mate infusions can be considered safe with respect to Cd and Pb available for human intake. These results corroborate with other studies that have evaluated the risk of Cd and Pb intake via plant foods from South America (Barbosa et al., 2019; Milani et al., 2019).

2.6.4 Cd AND Pb CONCENTRATION IN YERBA MATE LEAVES AND LEGISLATION

The overall mean values of Cd in yerba mate leaves (0.29 ± 0.30 mg kg^{-1}) corroborate previous studies: 0.37 ± 0.19 mg kg^{-1} (Valduga et al., 2019a), 0.30 ± 0.02 mg kg^{-1} (Schmite et al., 2019), 0.57 mg kg^{-1} (0.25 – 0.77 mg kg− 1) (Milani et al., 2019), 0.015 to 0.15 mg kg^{-1} (Santos et al., 2017), 0.20 ± 0.20 mg kg^{-1} (Barbosa et al., 2018), and 0.29 mg kg^{-1} (0.09− 0.84 mg kg^{-1}) (Motta et al., 2020). These values are often close to or above the limit established by Mercosul legislation – 0.40 mg kg^{-1} (Brasil, 2013).

Therefore, the limit established by Mercosul legislation (Brasil, 2013) is not consistent with the natural concentration of Cd in yerba mate leaves. A similar case was reported for cacao, where the new legislation implemented by the European

Union underestimated the natural Cd concentration in cacao seeds (Argüello et al., 2019). In this case, the new legislation also threatens the sustainability of the production chain in question.

The presence of Cd in the leaves of other plant species used for infusion has recently been reported by Milani et al. (2019), who have detected the presence of Cd in boldo leaf (*Pneumus boldus* Molina), peppermint (*Mentha piperita* L.), and in flowers of chamomile (*Matricaria recutita* L.). Cadmium concentration in the leaves and flowers ranged from 0.01 – 0.05 mg kg^{-1}, 0.09 – 0.25 mg kg^{-1}, and 0.03 – 0.08 mg kg^{-1}, respectively. Although grown under different conditions of soil and climate, other studies have shown that *C. sinensis* has a lower concentration of Cd than that of yerba mate leaves. Concentrations of Cd in C. sinensis ranged from 0.02 to 0.06 mg kg^{-1} (Zhao et al., 2017), 0.03 – 0.16 mg kg^{-1} (Marcos et al., 1998), and average of 0.10 mg kg^{-1} (Seenivasan et al., 2016). However, there are no limits for Cd concentration in tea in Japan (Tsushida and Takeo, 1977), China (Bugang and Woolsey, 2010), India (PFA, 2003) and in the European Union (EUROPEA, 2014). Nevertheless, yerba mate leaves seem to have the highest natural concentration of Cd when compared to other teas used for infusion drinks. Therefore, future studies under controlled conditions are needed to deepen this knowledge.

More recent works have reported Pb concentration in commercial yerba mate leaves at 0.34 ± 0.21 mg kg^{-1} (Valduga et al., 2019a), 0.39 ± 0.04 mg kg^{-1} (Shmite et al., 2019), 0.14 – 0.82 mg kg^{-1} (Milani et al., 2019), 0.18–1.25 mg kg^{-1} (Santos et al., 2017), 0.22 – 0.69 mg kg^{-1} (Barbosa et al., 2018) and 0.26 (below LOD to 0.60 mg kg^{-1}) (Motta et al., 2020). The average Pb concentration found in the present study was higher than the reported concentration values. However, the present study has covered a large variation in genetic of yerba mate plants, soil characteristics, and climate conditions. Genetic variation can be a factor responsible for this variation, as reported by Barbosa et al. (2018).

Studies have indicated that *C. sinensis* leaves have a higher Pb concentration than yerba mate leaves, with mean values of 2.41 mg kg^{-1} (Seenivasan et al., 2016), 1.92 mg kg^{-1} (Zheng et al., 2014), and 4.55 mg kg^{-1} (Jin et al., 2005). Milani et al. (2019) has reported the presence of Pb in leaves of boldo (*P. boldus*), peppermint (*M. piperita*) and in flowers of chamomile (*M. recutita*). In these plant parts Pb concentration was higher than Cd whose concentration was 0.15 mg kg^{-1} (0.06 – 0.25 mg kg^{-1}), 0.35 mg kg^{-1} (0.16 – 0.62 mg kg^{-1}) and 0.71 mg kg^{-1}

(0.54 – 0.95 mg kg^{-1}), respectively. Considering the herbal species cited, the limit established for Pb (0.60 mg kg^{-1}) by Mercosul legislation (Brasil, 2013) underestimate the natural Pb concentration in plant tissues, including yerba mate leaves.

If we take *C. sinensis* as an example, reported results have indicated high levels of Pb. However, in Asian legislations the Pb limits are much higher than that established for the Mercosul area, which is 2.5 mg kg^{-1} in Japan (Tsushida and Takeo, 1977), 5 mg kg^{-1} in China (Bugang and Woolsey, 2010) and 10 mg kg^{-1} in India (PFA, 2003), values that follow the natural Pb levels in *C. sinensis*. Thus, it is clear that the limits established by Mercosul legislation for Pb and Cd are very restricted and underestimate the natural concentrations of these elements in plants as confirmed in this study.

2.7 CONCLUSION

The pseudototal Cd and Pb concentrations in soil are dependent on the parent material. However, concentrations of Cd and Pb in yerba mate leaves were not related to soil type. The available concentration of Cd in soil was a good indicator for the presence of this metal in plant tissue. The main factors associated with Cd concentration in yerba mate leaves are cultivation on extremely acidic soils, Cd concentration in the respective soil, and the synergistic effect of Zn and Cd in the plant. Crop system was not associated with the elements determined in this study and yerba mate plants grown under natural conditions had Cd and Pb concentrations in leaves above the limit set by current legislation in South America. Cadmium and Pb occur naturally in yerba mate leaves and the exposure of these elements to consumers of yerba mate infusions is very low. The set limit levels for Cd and Pb in yerba mate leaves can affect trade rather than consumer health due to unrealistic legislation in South America. It can seriously harm the sustainability of yerba mate leaves production for infusion and it especially applies to Cd concentration in Rio Grande do Sul state (Brazil) and to Pb in all the surveyed countries.

2.8 REFERENCES

Alexander, P., Alloway, B. & Dourado, A. (2006). Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. *Environment Pollution*, 144 (3), 736–745.

Althaus, D., Gianello, C., Tedesco, M. J., Silva, K. J. D., Bissani, C. A. & Felisberto, R. (2018). Natural fertility and metals contents in soils of Rio Grande Do Sul (Brazil). *Revista Brasileira de Ciência do Solo*, p. 42.

Argüello, D., Chavez, E., Lauryssen, F., Vanderschueren, R., Smolders, E., & Montalvo, D. (2019). Soil properties and agronomic factors affecting cadmium concentrations in cacao beans: A nationwide survey in Ecuador. *Science of the total Environment*, 649, 120-127.

Barbosa, J. Z., dos Santos Domingues, C. R., Poggere, G. C., Motta, A. C. V., Dos Reis, A. R., de Moraes, M. F., & Prior, S. A. (2019). Elemental composition and nutritional value of Araucaria angustifolia seeds from subtropical Brazil. *Journal of food science and technology*, 56(2), 1073-1077.

Barbosa, J. Z., Motta, A. C., Consalter, R., Poggere, G. C., Santin, D., & Wendling, I. (2018). Plant growth, nutrients and potentially toxic elements in leaves of yerba mate clones in response to phosphorus in acid soils. *Anais da Academia Brasileira de Ciências*, 90(1), 557-571.

Barbosa, J. Z., Motta, A. C. V., dos Reis, A. R., Corrêa, R. S., & Prior, S. A. (2020). Spatial distribution of structural elements in leaves of *Ilex paraguariensis*: physiological and ecological implications. *Trees*, 34(1), 101-110.

Barbosa, J. Z., Zambon, L. M., Motta, A. C. V. & Wendling, I. (2015). Composition, hot-water solubility of elements and nutritional value of fruits and leaves of yerba-mate. *Ciência e Agrotecnologia,* 39(6), 593–603.

Bewick, V., Cheek, L., & Ball, J. (2004). Statistics review 10: further nonparametric methods. *Critical care*, 8(3), 1-4.

Brasil. (2013). Dispõe sobre o Regulamento Técnico MERCOSUL sobre Limites Máximos de Contaminantes Inorgânicos em Alimento (Resolução-RDC nº 42, de 29 de agosto de 2013). *Diário Oficial [da] República Federativa do Brasil.*

Bugang, W., & Woolsey, M. (2010). National food safety standardmaximum levels of contaminants in food. *People's Republic of china. Gain Report Number-CH10000.*

Europea (2014). U. Reglamento (UE) No 488/2014 de la Comision, de 12 de mayo de 2014, que modifica el Reglamento (CE) No 1881/2006 por lo que respecta al contenido máximo de cadmio en los productos alimenticios, 138. Diario Oficial de la Union Europea L, p. 13.

Frigo, C., Magri, E., Barbosa, J. Z., Sarteretto, L. M., Araujo, E. M., Prior, S. A., & Motta, A. C. V. (2020). Influence of roadways on heavy metal content in soils and yerba mate tissue in southern Brazil. *Management of Environmental Quality: An International Journal,* 31 No. 6, pp. 1477-1495. https://doi.org/10.1108/MEQ-10-2019-0219.

Gaw, S. K., Kim, N. D., Northcott, G. L., Wilkins, A. L., & Robinson, G. (2008). Uptake of ΣDDT, arsenic, cadmium, copper, and lead by lettuce and radish grown in contaminated horticultural soils. *Journal of agricultural and food chemistry*, 56(15), 6584-6593.

Grotz, N. & Guerinot, M. L. (2006). Molecular aspects of Cu, Fe and Zn homeostasis in plants*. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1763(7), 595-608.

Gupta, D. K., Chatterjee, S., Datta, S., Veer, V. & Walther, C. (2014). *Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals*. Chemosphere, 108, 134-144.

Han, W. Y., Zhao, F. J., Shi, Y. Z., Ma, L. F. & Ruan, J. Y. (2006). Scale and causes of lead contamination in Chinese tea. *Environmental Pollution*, 139(1), 125-132.

Holmgren, G. G. S., Meyer, M. W., Chaney, R. L. & Daniels, R. B. (1993). Cadmium, lead, zinc, copper, and nickel in agricultural soils of the United States of America. *Journal of Environmental Quality*, 22 (2), 335–348.

Hothorn, T., Hornik, K. & Zeileis, A. (2006). Unbiased recursive partitioning: a conditional inference framework. *Journal of Computational and Graphical statistics*. Stat. 15 (3), 651–674.

Inmetro - Instituto Nacional de Metrologia and Normalização e Qualidade Industrial. (2020). DOQ-CGCRE-008, Orientações sobre validação de métodos de ensaios químicos.

Jin, C. W., Zheng, S. J., He, Y. F., Zhou, G. D. & Zhou, Z. X. (2005). Lead contamination in tea garden soils and factors affecting its bioavailability. *Chemosphere*, 59, 1151–1159.

Joint FAO/WHO Expert Committee on Food Additives. (2010). Joint FAO/WHO Expert Committee on Food Additives Seventy-third Meeting. World Health Organization, Geneva.

Kabata-Pendias, A. (2010). Trace elements in soils and plants, 4th ed. CRC Press, Pulawy, Poland.

Kaiser, H.F. & Rice, J. (1974). Little jiffy, mark IV. *Educational and psychological measurement*, 34 (1), 111–117.

Kidd, P., Mench, M., Alvarez-Lopez, V., Bert, V., Dimitriou, I., Friesl-Hanl, W., Herzig, R., Janssen, J. O., Kolbas, A., Muller, I., Neu, S., Renella, G., Ruttens, A., Vangronsveld, J. & Puschenreiter, M. (2015). Agronomic practices for improving gentle remediation of trace element-contaminated soils. *International journal of phytoremediation*, 17 (11), 1005–1037.

Ma, C., Liu, F. Y., Hu, B., Wei, M. B., Zhao, J. H., Zhang, K. & Zhang, H. Z. (2019). Direct evidence of lead contamination in wheat tissues from atmospheric deposition based on atmospheric deposition exposure contrast tests. *Ecotoxicological and Environtal Safety*, 185, 109688.

Magri, E., Gugelmin, E. K., Grabarski, F. A. P., Barbosa, J. Z., Auler, A. C., Wendling, I., Prior, S. A., Valduga, A. T., Motta, A. C. V., 2020. Manganese hyperaccumulation capacity of *Ilex paraguariensis* A. St. Hil. and occurrence of interveinal chlorosis induced by transient toxicity. Ecotoxicology and Environmental Safety, 203, 111010.

Marcelo, M. C. A., Martins, C. A., Pozebon, D., Dressler, V. L. & Ferrão, M. F. (2014). Classification of yerba mate (*Ilex paraguariensis*) according to the country of origin based on element concentrations. *Microchemical Journal*, 117, 164–171.

Marcos, A., Fisher, A., Rea, G. & Hill, S. J. (1998). Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *Journal of Analytical Atomic Spectrometry*. 13 (6), 521–525.

Marques, A. C. (2014). As paisagens do mate e a conservação socioambiental: um estudo junto aos agricultores familiares do planalto norte catarinense (434 p. (Tese, curso de Doutorado em Meio Ambiente e Desenvolvimento da Universidade Federal do Paraná)).

Marques, R. & Motta, A. C. V. (2003). Análise química do solo para fins de fertilidade. In: Lima, M.R.de. (Ed.), *Manual de diagnóstico da fertilidade e manejo* *dos solos agrícolas, 2nd ed.* Departamento de Solos e Engenharia Agrícola, Curitiba, pp. 81–102.

Martınez-Villegas, N., Flores-V´elez, L. M. & Domınguez, O. (2004). Sorption of lead in soil as a function of pH: a study case in México. *Chemosphere* 57 (10), 1537–1542.

Mendoza, J. (2020). Latin America: yerba mate per capita consumption 2018, by country. Available in:https://www.statista.com/statistics/884925/consumption-yerba-mateper-capita-latin-america/#statisticContainerAccessed: September 10, 2020.

Milani, R. F., Silvestre, L. K., Morgano, M. A. & Cadore, S. (2019). Investigation of twelve trace elements in herbal tea commercialized in Brazil. *Journal of Trace Elements in Medicine and Biology*, 52, 111–117.

Mitra, G. N. (2015). Regulation of Nutrient Uptake by Plants Gyanendra Nath Mitra A Biochemical and Molecular Approach. Springer.

Molitor, M., Dechamps, C., Gruber, W. & Meerts, P. (2005). Thlaspi caerulescens on nonmetalliferous soil in Luxembourg: ecological niche and genetic variation in mineral element composition. *New Phytol*ogist, 165 (2), 503–512.

Moreno-Jiménez, E., Fernandez, J. M., Puschenreiter, M., Williams, P. N. & Plaza, C. (2016). Availability and transfer to grain of As, Cd, Cu, Ni, Pb and Zn in a barley agri-system: impact of biochar, organic and mineral fertilizers. *Agriculture, Ecosystems & Environment*, 219, 171–178.

Motta, A. C. V., Barbosa, J. Z., Magri, E., Pedreira, G. Q., Santin, D., Prior, S. A., Consalter, R., Young, S. D., Broadley, M. R. & Benedetti, E. L. (2020). Elemental composition of yerba mate (*Ilex paraguariensis* A. St.-Hil.) under low input systems of southern Brazil. *Science of the Total Environment,* 736, 139637.

PFA. (2003). Prevention of Food Adulteration Act of India, 1954 and Rules. *Eastern Book Company*, Lucknow, India.

Poletto, I., Muniz, M. F. B., Ceconi, D. E. & Blume, E. (2011). Influência da aplicação de NPK e calcário sobre o crescimento da erva-mate, severidade da podridão-de-raízes e população fúngica do solo. *Ciência Florestal*, 21 (3), 429–444.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Rabel, D. O., Motta, A. C. V., Barbosa, J. Z., Melo, V. F. & Prior, S. A., 2018. Depth distribution of exchangeable aluminum in acid soils: a study from subtropical Brazil. *Acta Scientiarum,* 40, e39320.

Reeves, R. D., Van Der Ent, A. & Baker, A. J. (2018). Global distribution and ecology of hyperaccumulator plants. In: *Van Der Ent,* A., Echevarria, G., Baker, A.J.M., Morel, J.L. (Ed.), Agromining: Farming for Metals. Springer, pp. 75–92. https://doi.org/10.1007/978-3-319-61899-9_5.

Rizwan, M., Ali, S., Adrees, M., Ibrahim, M., Tsang, D. C., Rehman, M. Z., Zahir, Z. A., Rinklebe, J., Tack, F. M. & Ok, Y. S. (2017)a. A critical review on effects, tolerance mechanisms and management of cadmium in vegetables. *Chemosphere*, 182, 90–105.

Rizwan, M., Ali, S., Hussain, A., Ali, Q., Shakoor, M. B., Zia-ur-Rehman, M., Farid, M. & Asma, M. (2017)b. Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. *Chemosphere,* 187, 35–42. https://doi.org/10.1016/j.chemosphere.2017.08.071.

Roberts, T. L. (2014). Cadmium and phosphorous fertilizers: the issues and the science. *Procedia Engineering,* 83, 52–59.

Santin, D., Benedetti, E. L. & Reissmann, C. B. (2015). Nutrição e recomendação de adubação e calcário para a cultura da erva-mate. In: Wendling, I., Santin, D. (Eds.), *Propagação e nutrição de erva-mate*, pp. 99–195. Brasília: Embrapa.

Santos, L. M. G. D., Neto, V., Alves, S., Iozzi, G. & Jacob, S. D. C. (2017). Arsenic, cadmium and lead concentrations in Yerba mate commercialized in Southern Brazil by inductively coupled plasma mass spectrometry. *Ciência Rural*, 47 (12).

Schmite, B. D. F. P., Bitobrovec, A., Hacke, A. C. M., Pereira, R. P., Los Weinert, P. & dos Anjos, V. E. (2019). In vitro bioaccessibility of Al, Cu, Cd, and Pb following simulated gastro-intestinal digestion and total content of these metals in different Brazilian brands of yerba mate tea. *Food Chemistry*, 281, 285–293.

Seenivasan, S., Anderson, T. A. & Muraleedharan, N. (2016). Heavy metal content in tea soils and their distribution in different parts of tea plants, *Camellia sinensis* (L). O. Kuntze. *Environmental monitoring and assessment*, 188 (7), 428.

Škrbić, B. & Čupić, S. (2005). Toxic and essential elements in soft wheat grain cultivated in Serbia. *European Food Research and Technology*, 221 (3), 361–366.

Škrbić, B. & Cvejanov, J. (2011). The enrichment of wheat cookies with high-oleic sunflower seed and hull-less barley flour: impact on nutritional composition, content of heavy elements and physical properties. *Food Chemestry*, 124 (4), 1416–1422.

Stafilov, T., Škrbić, B., Klánová, J., Čupr, P., Holoubek, I., Kočov, M. & Đurišić-Mladenović, N. (2011). Chemometric assessment of the semivolatile organic contaminants content in the atmosphere of the selected sites in the Republic of Macedonia. *Journal of chemometrics*, 25 (5), 262–274.

Toppel, F. V., Junior, A. M., Motta, A. C. V., Frigo, C., Magri, E. & Barbosa, J. Z. (2018). Soil chemical attributes and their influence on elemental composition of yerba mate leaves. *Floresta*, 48(3), 425-434.

Troadec, M. B., Ward, D. M., Lo, E., Kaplan, J. & De Domenico, I. (2010). Induction of FPN1 transcription by MTF-1 reveals a role for ferroportin in transition metal efflux. *Blood, The Journal of the American Society of Hematology*,116 (22), 4657–4664.

Tsushida, T. & Takeo, T. (1977). Zinc, copper, lead and cadmium contents in green tea. *Journal of the Science of Food and Agriculture*, 28 (3), 255–258.

Usepa, E. (1995). Method 3051: Microwave assisted acid digestion of sediments, sludges, soils, and oils. *Test methods for evaluating solid waste*, pp 1-30.

Valduga, A. T., Gonçalves, I. L. & Magri, E., (2019)a. Analysis of the presence of toxic metals in yerba mate samples: a case study from South Brazil. *Water Air Soil Pollut*. 230 (7), 153.

Valduga, A. T., Gonçalves, I. L., Magri, E. & Finzer, J. R. D. (2019)b. Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. *Food Research International*. Int. 120, 478–503.

Wang, Q., Xie, Z. & Li, F. (2015). Using ensemble models to identify and apportion heavy metal pollution sources in agricultural soils on a local scale. *Environmental Pollution*, 206, 227–235.

Werkenthin, M., Kluge, B. & Wessolek, G. (2014). Metals in European roadside soils and soil solution–a review*. Environmental Pollution*. 189, 98–110.

Whitley, E. & Ball, J. (2002). Statistics review 6: nonparametric methods. *Critical Care*, 6 (6), 509.

Yang, Y., Chang, A. C., Wang, M., Chen, W. & Peng, C. (2018). Assessing cadmium exposure risks of vegetables with plant uptake factor and soil property. *Environmental Pollution*, 238, 263–269.

Yaylalı-Abanuz, G. & Tüysüz, N. (2009). Heavy metal contamination of soils and tea plants in the eastern Black Sea region, NE Turkey. *Environmental Earth Science*, 59 (1), 131.

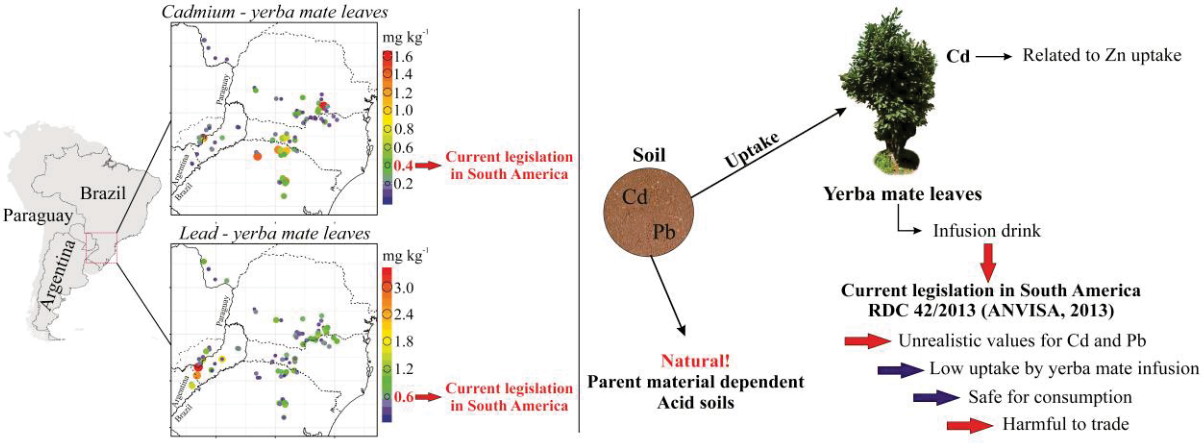
Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F. & Zhang, G. (2011). The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Environmental Pollution*, 159 (1), 84–91.

Zhao, H., Zhang, S. & Zhang, Z. (2017). Relationship between multi-element composition in tea leaves and in provenance soils for geographical traceability. *Food Control,* 76, 82–87.

Zheng, H., Li, J. L., Li, H. H., Hu, G. C. & Li, H. S. (2014). Analysis of trace metals and perfluorinated compounds in 43 representative tea products from South China. *Journal of Food Science*, 79 (6), 1123–1129.

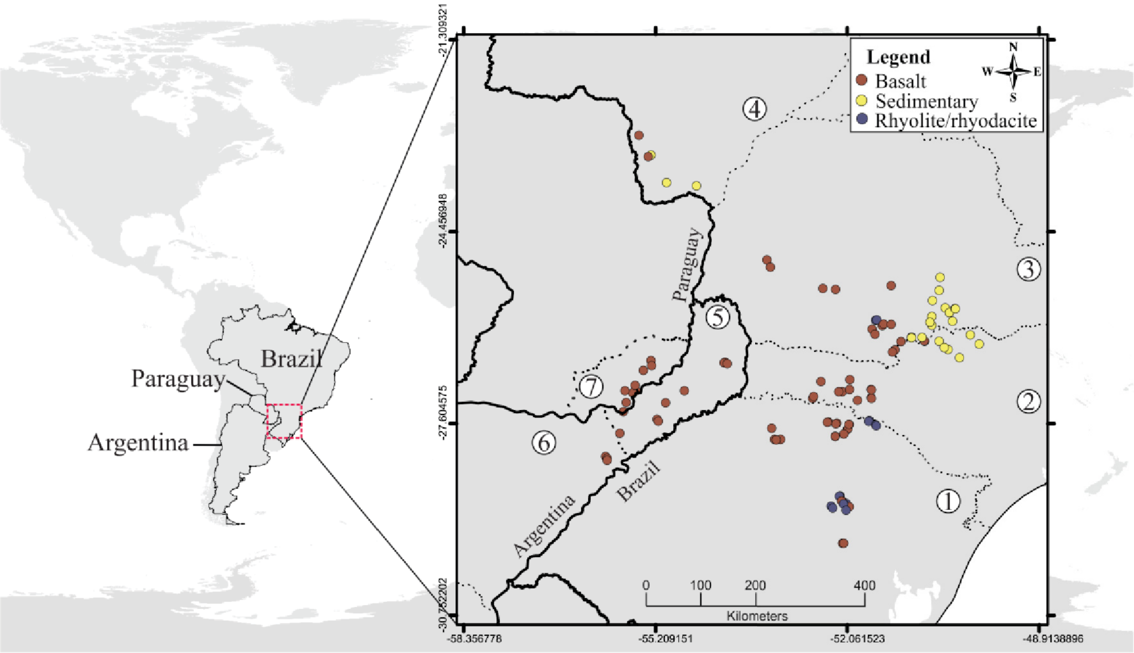
2.9 SUPPLEMENTARY MATERIAL

GRAPHICAL ABSTRACT



SOURCE: The author (2021).

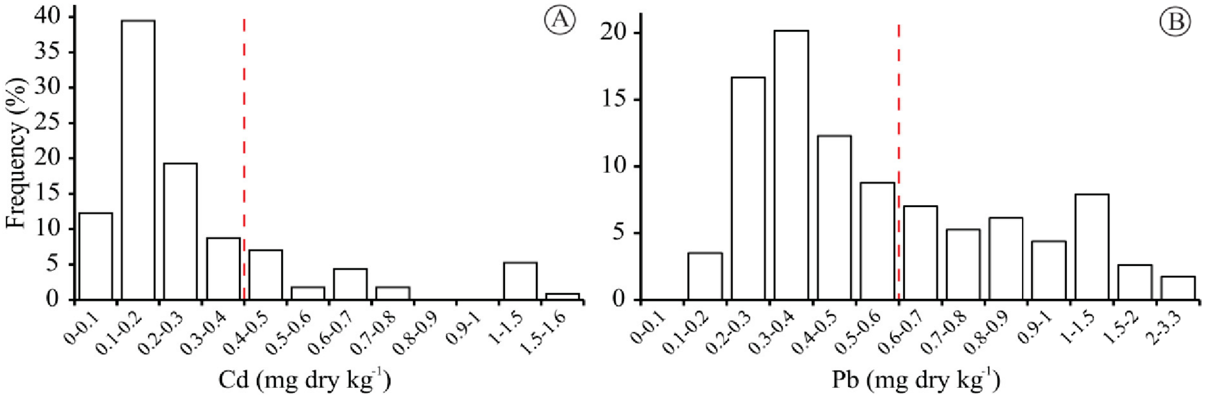
FIGURE S1 – GEOGRAPHIC LOCALIZATION OF THE 115 SAMPLING SITES, WHICH REPRESENT THE MAIN YERBA MATE PRODUCING REGIONS IN SOUTH AMERICA. SOLID LINES DEFINE THE BORDERLINES BETWEEN COUNTRIES AND DASHED LINES REPRESENT THE LIMITS BETWEEN STATES, PROVINCES, AND DEPARTMENTS WITHIN COUNTRIES.



SOURCE: The author (2021).

LEGEND: (1) *Rio Grande do Sul*-BR; (2) *Santa Catarina*-BR; (3) *Paraná*-BR; (4) *Mato Grosso do Sul*-BR; (5) *Missiones*-AR; (6) *Corrientes*-AR; (7) *Itapuá*-PY.

FIGURE S2 – HISTOGRAM OF Cd (A) AND Pb (B) CONCENTRATIONS FOR THE 115 SAMPLES OF YERBA MATE LEAVES. DASHED RED LINE REFERS TO THE LEGAL LIMITS FOR CD AND PB IN YERBA MATE LEAVES IN SOUTH AMERICA.



SOURCE: The author (2021).

TABLE S1 – SOIL SAMPLES INFORMATION (N=115): MUNICIPALITY, COUNTRY, GEOGRAPHICAL COORDINATES, SOIL TYPE, AND MANAGEMENT SYSTEM. (to be continued)

Site	Municipality/state[1]	Country[2]	Latitude	Longitude	Soil[3]	System[4]	Fertilizer	Herbicide
1	Barão de Cotegipe/RS	BR	-27.577831	-52.382768	Bas.	Plan.	Yes	Yes
2	Barão de Cotegipe/RS	BR	-27.577761	-52.380311	Bas.	Agro.	Yes	Yes
3	Erebango/RS	BR	-27.817170	-52.265143	Bas.	Plan.	No	Yes
4	Erebango/RS	BR	-27.810520	-52.261680	Bas.	Plan.	No	Yes
5	Áurea/RS	BR	-27.688528	-52.055887	Bas.	Plan.	Yes	No
6	Áurea/RS	BR	-27.701582	-52.065254	Bas.	Plan.	Yes	No
7	Erechim/RS	BR	-27.601770	-52.236138	Bas.	Plan.	Yes	Yes
8	Erechim/RS	BR	-27.608952	-52.254917	Bas.	Agro.	Yes	No
9	Machadinho/RS	BR	-27.557461	-51.708615	Rhy.	Plan.	No	Yes
10	Machadinho/RS	BR	-27.560745	-51.710918	Rhy.	Agro.	No	Yes
11	Barracão/RS	BR	-27.627123	-51.599761	Rhy.	Agro.	No	No
12	Viadutos/RS	BR	-27.619292	-52.037670	Bas.	Plan.	Yes	Yes
13	Viadutos/RS	BR	-27.619563	-52.036759	Bas.	Agro.	No	Yes
14	Ilópolis/RS	BR	-28.911375	-52.130104	Rhy.	Plan.	Yes	Yes
15	Ilópolis/RS	BR	-28.922772	-52.127374	Rhy.	Agro.	No	No
16	Ilópolis/RS	BR	-28.919884	-52.080592	Bas.	Plan.	Yes	Yes
17	Anta gorda/RS	BR	-28.970959	-52.034065	Bas.	Plan.	Yes	Yes
18	Arvorezinha/RS	BR	-28.877593	-52.157643	Rhy.	Plan.	Yes	Yes
19	Arvorezinha/RS	BR	-28.880155	-52.141707	Rhy.	Agro.	No	No
20	Novo Barreiro/RS	BR	-27.863751	-53.160082	Bas.	Agro.	No	No
21	Arvorezinha/RS	BR	-28.876994	-52.146779	Rhy.	Plan.	Yes	Yes
22	Itapuca /RS	BR	-28.795922	-52.194201	Rhy.	Plan.	Yes	Yes
23	Itapuca /RS	BR	-28.794926	-52.187708	Rhy.	Agro.	No	No
24	Fontoura Xavier/RS	BR	-28.959814	-52.332470	Rhy.	Plan.	No	Yes
25	Fontoura Xavier/RS	BR	-28.989077	-52.305283	Rhy.	Agro.	No	No
26	Putinga/RS	BR	-29.026792	-52.085537	Rhy.	Plan.	Yes	Yes
27	Venâncio Aires/RS	BR	-29.567353	-52.143673	Bas.	Plan.	Yes	Yes
28	Venâncio Aires/RS	BR	-29.570459	-52.134531	Bas.	Plan.	No	Yes
29	Palmeira das Missões/RS	BR	-27.872578	-53.239530	Bas.	Plan.	No	Yes
30	Palmeira das Missões/RS	BR	-27.862645	-53.249826	Bas.	Agro.	No	No
31	Boa vista das Missões/RS	BR	-27.683554	-53.308221	Bas.	Plan.	No	Yes
32	Concórdia/SC	BR	-27.222643	-51.900442	Bas.	Plan.	No	Yes
33	Ipumirim/SC	BR	-27.085413	-52.148839	Bas.	Plan.	No	Yes
34	Ipumirim/SC	BR	-27.085144	-52.204201	Bas.	Plan.	No	Yes
35	Lindóia do Sul/SC	BR	-27.048175	-52.027032	Bas.	Plan.	No	Yes
36	Jaborá/SC	BR	-27.192774	-51.671444	Bas.	Plan.	No	No
37	Jaborá/SC	BR	-27.184369	-51.674539	Bas.	Agro.	No	No
38	Ponte Serrada/SC	BR	-26.887388	-52.025501	Rhy.	Agro.	No	No
39	Catanduvas/SC	BR	-27.053805	-51.672127	Bas.	Plan.	Yes	Yes
40	Catanduvas/SC	BR	-27.053208	-51.671647	Bas.	Agro.	No	No
41	Catanduvas/SC	BR	-27.048408	-51.674480	Bas.	Agro.	No	No
42	Chapecó/SC	BR	-27.190411	-52.616258	Bas.	Plan.	No	Yes
43	Chapecó/SC	BR	-27.190367	-52.620175	Bas.	Agro.	No	No
44	Xaxim/SC	BR	-26.916790	-52.500288	Bas.	Plan.	No	Yes
45	Canoinhas /SC	BR	-26.257082	-50.559100	Sed.	Agro.	No	No
46	Mafra/SC	BR	-26.150534	-50.047056	Sed.	Agro.	No	No
47	Irineópolis/SC	BR	-26.249553	-50.805391	Bas.	Plan.	No	No
48	Irineópolis/SC	BR	-26.252850	-50.801065	Bas.	Agro.	No	No
49	Major Vieira/SC	BR	-26.388499	-50.415275	Sed.	Agro.	No	No
50	Bela vista do Toldo/SC	BR	-26.358527	-50.474019	Sed.	Agro.	No	No
51	Monte Castelo/SC	BR	-26.527963	-50.227048	Sed.	Agro.	No	No
52	Itaiópolis/SC	BR	-26.299360	-49.904457	Sed.	Agro.	No	No
53	Cascavel/PR	BR	-24.922581	-53.383568	Bas.	Agro.	No	Yes
54	Cascavel/PR	BR	-25.039509	-53.327834	Bas.	Agro.	No	No
55	Porto Vitória/PR	BR	-26.262034	-51.185200	Bas.	Agro.	No	No
56	União da Vitória/PR	BR	-26.195513	-51.014873	Sed.	Plan.	No	No
57	União da Vitória/PR	BR	-26.188665	-51.011253	Sed.	Agro.	No	No

SOURCE: The author (2021).

LEGEND: [1]RS: Rio Grande do Sul, PR: Paraná, SC: Santa Catarina, MS: Mato Grosso do Sul, MI: Missiones, CO: Corrientes, IT: Itapuá. [2]BR: Brazil, AR: Argentina, PY: Paraguay. [3]Bas: Basalt, Rhy: Rhyolite/rhyodacite, Sed: Sedimentary. [4]Pant: Plantation, Agro: Agroforest.

TABLE S1 – SOIL SAMPLES INFORMATION (N=115): MUNICIPALITY, COUNTRY, GEOGRAPHICAL COORDINATES, SOIL TYPE, AND MANAGEMENT SYSTEM. (conclusion)

Site	Municipality/state[1]	Country[2]	Latitude	Longitude	Soil[3]	System[4]	Fertilizer	Herbicide
58	Guarapuava /PR	BR	-25.342595	-51.347239	Bas.	Plan.	No	Yes
59	Paula Freitas/PR	BR	-26.192040	-50.839488	Sed.	Plan.	No	Yes
60	Pinhão/PR	BR	-25.904767	-51.587382	Rhy.	Plan.	No	No
61	Pinhão/PR	BR	-25.913171	-51.599611	Rhy.	Agro.	No	No
62	General Carneiro /PR	BR	-26.395435	-51.282674	Bas.	Plan.	No	No
63	General Carneiro/PR	BR	-26.433833	-51.326109	Bas.	Agro.	No	No
64	Paulo Frontin/PR	BR	-25.995248	-50.678194	Sed.	Agro.	No	No
65	Fernandes Pinheiro/PR	BR	-25.421365	-50.560621	Sed.	Plan.	No	No
66	Rebouças/PR	BR	-25.585345	-50.667440	Sed.	Plan.	No	Yes
67	Rebouças/PR	BR	-25.709434	-50.462998	Sed.	Agro.	No	No
68	Bituruna /PR	BR	-26.058122	-51.657240	Bas.	Plan.	No	No
69	Bituruna /PR	BR	-26.140243	-51.611603	Bas.	Agro.	No	No
70	Imbituva/PR	BR	-25.207627	-50.544169	Sed.	Plan.	Yes	Yes
71	Imbituva/PR	BR	-25.208512	-50.542895	Sed.	Agro.	No	No
72	São Matheus do Sul/PR	BR	-25.924773	-50.342100	Sed.	Plan.	Yes	Yes
73	São Matheus do Sul/PR	BR	-25.783368	-50.395258	Sed.	Agro.	No	No
74	Rio Azul/PR	BR	-25.846749	-50.677299	Sed.	Agro.	No	No
75	São Jõao do Triunfo/PR	BR	-25.721733	-50.295853	Sed.	Agro.	No	No
76	São Jõao do Triunfo/PR	BR	-25.718260	-50.298294	Sed.	Agro.	No	No
77	Mallet/PR	BR	-25.943562	-50.711190	Sed.	Agro.	No	No
78	Laranjeira do Sul/PR	BR	-25.392230	-52.466581	Bas.	Agro.	No	No
79	Virmond/PR	BR	-25.404100	-52.256076	Bas.	Plan.	No	No
80	Cruz Machado /PR	BR	-25.975037	-51.469317	Bas.	Plan.	Yes	Yes
81	Cruz Machado /PR	BR	-25.975037	-51.469317	Bas.	Plan.	No	Yes
82	Cruz Machado /PR	BR	-26.001260	-51.493468	Bas.	Agro.	No	No
83	Cruz Machado /PR	BR	-25.977830	-51.347244	Bas.	Agro.	No	No
84	Aral Moreira/MS	BR	-22.880087	-55.491871	Bas.	Agro.	Yes	Yes
85	Aral Moreira/MS	BR	-22.884178	-55.523545	Bas.	Plan.	Yes	Yes
86	Amambaí/MS	BR	-23.208604	-55.313541	Sed.	Plan.	Yes	Yes
87	Amambaí/MS	BR	-23.217295	-55.326664	Bas.	Agro.	No	No
88	Tacurú/MS	BR	-23.653623	-55.035714	Sed.	Plan.	Yes	Yes
89	Iguatemi/MS	BR	-23.706483	-54.548374	Sed.	Agro.	No	No
90	Guaraní/MI	AR	-27.537227	-55.195176	Bas.	Plan.	Yes	Yes
91	Guaraní/MI	AR	-27.566066	-55.171722	Bas.	Plan.	No	No
92	San José/MI	AR	-27.767257	-55.803229	Bas.	Plan.	Yes	Yes
93	Gobernador Virasoro/CO	AR	-28.144419	-56.036905	Bas.	Plan.	Yes	Yes
94	Gobernador Virasoro/CO	AR	-28.176751	-56.011006	Bas.	Plan.	Yes	Yes
95	Santo Tomé/CO	AR	-28.210295	-56.008667	Bas.	Plan.	Yes	Yes
96	Campo Viera/MI	AR	-27.266022	-55.050853	Bas.	Plan.	Yes	Yes
97	Cainguás/MI	AR	-27.066031	-54.744057	Bas.	Plan.	Yes	Yes
98	Cainguás/MI	AR	-27.065227	-54.742477	Bas.	Agro.	No	No
99	San Pedro/MI	AR	-26.610123	-54.094308	Bas.	Plan.	Yes	Yes
100	San Pedro/MI	AR	-26.622323	-54.042971	Bas.	Plan.	Yes	Yes
101	San Pedro/MI	AR	-26.598809	-54.075227	Bas.	Plan.	Yes	Yes
102	Bella Vista Sur/IT	PY	-27.066617	-55.535270	Bas.	Agro.	No	No
103	Bella Vista Sur/IT	PY	-27.070441	-55.548737	Bas.	Plan.	Yes	Yes
104	Puerto Obligado/IT	PY	-27.103799	-55.587435	Bas.	Plan.	Yes	Yes
105	Encarnación/IT	PY	-27.413485	-55.744875	Bas.	Plan.	Yes	Yes
106	Nueva Alborada/IT	PY	-27.258668	-55.699500	Bas.	Plan.	Yes	Yes
107	Jesús de Tavarangue/IT	PY	-27.066590	-55.720084	Bas.	Plan.	Yes	Yes
108	Bella Vista Sur/IT	PY	-26.978081	-55.551779	Bas.	Plan.	Yes	Yes
109	María Auxiliadora/IT	PY	-26.570946	-55.290297	Bas.	Plan.	Yes	Yes
110	María Auxiliadora/IT	PY	-26.654085	-55.281509	Bas.	Plan.	Yes	Yes
111	Capitán Meza/IT	PY	-26.733506	-55.418179	Bas.	Plan.	Yes	Yes
112	Ilópolis/RS	BR	-28.933460	-52.111289	Rhy.	Agro.	No	No
113	Barracão/RS	BR	-27.631726	-51.591187	Rhy.	Agro.	No	No
114	Barracão/RS	BR	-27.634482	-51.588550	Rhy.	Plan.	No	Yes
115	Erebango/RS	BR	-27.776538	-52.123852	Bas.	Plan.	No	Yes

SOURCE: The author (2021).

LEGEND: [1]RS: Rio Grande do Sul, PR: Paraná, SC: Santa Catarina, MS: Mato Grosso do Sul, MI: Missiones, CO: Corrientes, IT: Itapuá. [2]BR: Brazil, AR: Argentina, PY: Paraguay. [3]Bas: Basalt, Rhy: Rhyolite/rhyodacite, Sed: Sedimentary. [4]Pant: Plantation, Agro: Agroforest.

TABLE S2 – GFAAS HEATING AND TEMPERATURE PROGRAM DEVELOPED FOR CD AND PB DETERMINATION IN YERBA MATE LEAVES.

Procedure	**Temperature (ºC)**		**Time (s)**		**Heating mode**	**Air flow rate (L min^{-1})**
	Pb	Cd	Pb	Cd		
Drying	120	50	100	20	Ramp	0.1
	200	120	20	90	Ramp	1.0
	-	200	-	20	Ramp	1.0
Pre-pyrolysis	700	550	10	10	Ramp	1.0
Pyrolysis	900	700	10	3	Hold	1.0
Atomization	1700	1600	2	2	Hold	0.0
Cleaning	2500	2500	2	3	Hold	1.0

SOURCE: The author (2021).

TABLE S3 – ICP OES INSTRUMENTAL PARAMETERS.

Power	1.2 kW
Plasma gas flow rate	15 L min^{-1}
Auxiliary gas flow rate	1.5 L min^{-1}
Nebulizer pressue	200 kPa
Nebulizer type	Concentric glass nebulizer, SeaSpray 1/p
Spray chamber	Cyclonic Spray Chamber
Replicate read time	1 s
Replicate	3
Torch type	Qtz Torch Body, Dem, Axial
Analytical lines	214.439 nm (Cd); 327.395 nm (Cu); 238.204 nm (Fe); 257.61 nm (Mn); 213.618 nm (P); 220.353 nm (Pb); 213.857 nm (Zn);

SOURCE: The author (2021).

TABLE S4 – CERTIFIED REFERENCE MATERIAL 2710A – *MONTANA I SOIL* ANALYSIS; CONCENTRATION IN mg kg^{-1}.

Element	**Value certified**	**Value obtained**	**Recovery (%)**	**LOD**	**LOQ**
Cd	12.3	13	106	0.03	0.1
Cu	3420	3286	96	0.19	0.63
Fe	43200	44436	103	0.21	0.7
Mn	2140	2099	98	0.02	0.07
P	1050	1009	96	2.05	6.83
Pb	5520	5450	99	0.01	0.03
Zn	4180	4306	103	0.21	0.7

SOURCE: The author (2021).

TABLE S5 – CERTIFIED REFERENCE MATERIAL (GBW–10016 *Camellia sinensis*) ANALYSIS; CONCENTRATION IN mg kg^{-1}.

Element	**Value certified**	**Value obtained**	**Recovery (%)**	**LOQ**	**LOD**	**Repeatability (%)**
Cd	0.076	0.086	113	0.015	0.051	8.68
Pb	1.6	1.66	104	0.005	0.016	1.92
Zn	35	31	89	0.44	1.48	7.67

SOURCE: The author (2021).

TABLE S6. PRINCIPAL COMPONENT ANALYSIS (PCA) FOR AVAILABLE Fe, Mn, Cu, Zn, Cd, AND Pb, AND PSEUDOTOTAL CONCENTRATIONS OF Fe, Mn, P, Cu, Zn, Cd, AND Pb IN SOILS (0 – 20 CM TOPSOIL).

Variable	**CP1**		**CP2**	
	Eigenvector	**σ^2 (%)**	**Eigenvector**	**σ^2 (%)**
Pseudototal concentrations (USEPA 3051a)				
Fe	0.44	19.20	0.04	0.15
Mn	0.39	15.44	-0.24	5.53
P	0.33	10.81	-0.03	0.07
Cu	0.41	17.20	0.20	4.13
Zn	0.42	17.99	-0.12	1.40
Pb	-0.05	0.27	-0.94	88.72
Cd	0.44	19.09	0.00	0.00
Eigenvalues	2.18		1.04	
% explained	67.8		15.5	
% accumulated	67.8		83.3	
Available concentrations (Mehlich-I)				
Mn	0.47	22.18	0.01	0.01
Fe	-0.42	17.45	0.34	11.37
Zn	0.37	14.05	0.55	29.72
Cu	0.36	12.92	-0.44	19.74
Cd	0.43	18.72	0.52	26.96
Pb	-0.38	14.67	0.35	12.20
Eigenvalues	1.64		1.19	
% explained	45		23.4	
% accumulated	45		68.4	

SOURCE: The author (2021).

3 CHAPTER II: LINKING EDAFOCLIMATIC CONDITIONS TO THE ELEMENTARY COMPOSITION OF THE YERBA MATE LEAVES IN SOUTH AMERICA

3.1 RESUMO

Estudos que avaliam a contribuição dos fatores edafoclimáticos na composição elementar da erva-mate são incipientes. A partir de amostras de folhas e solo tomadas nos principais polos produtores de erva-mate da América do Sul (n= 115), buscou-se identificar relações entre as condições de crescimento dos sítios amostrados com os teores elementares das folhas da erva-mate. Foi observada uma grande amplitude dos teores foliares de micronutrientes, Al e Ba, que no geral foram menores quando cultivado em solos formados por material sedimentar; e maiores quando cultivado em solos originados por basalto, especialmente do Paraguai e Rio Grande do Sul-BR. O teor de Mn foliar foi maior nas amostras de agroflorestas em relação aos monocultivos, e o oposto foi observado para o P. Também foi constatado correlação negativa entre o Mn foliar e o pH do solo; e correlação positiva entre o teor de B e Ca foliar e a temperatura média anual. Para as plantas cultivadas sob solos de basalto e riolito/riodacito, o teor de N foliar foi correlacionado com o teor de matéria orgânica do solo e com a altitude. Estes resultados indicam que as condições de crescimento em que a erva-mate é cultivada tem implicações na composição elementar de suas folhas, bem como na qualidade do produto gerado a partir delas.

Palavras-chave: *Ilex paraguariensis*. Origem geográfica. Nutrientes. Material de origem do solo. Abordagem multivariada.

3.2 ABSTRACT

Studies for assessing the contribution of edaphoclimatic factors to the elemental composition of yerba mate are incipient. In this study, we have identified relationships between soil conditions and climate with the elemental composition of yerba mate leaves collected from the main producing regions of yerba mate in South America. (n = 115). Results have shown great amplitude of micronutrients, Al, and Ba contents in leaves. Element contents in leaves were generally smaller when yerba mate was cultivated on soils originated from sedimentary material and higher when cultivated on soils originated from basalt, especially in Paraguay and Rio Grande do Sul-Brazil. Leaf content of Mn was higher in plants cultivated in agroforestry system than plants from monocultures, and the opposite was observed for P. There was also a negative correlation between Mn in leaves and soil pH, and a positive correlation between B and Ca in leaves and average annual temperature. N content in plant leaves grown on basaltic and rhyolite/rhyodacite soils correlated with soil organic matter and altitude. These results indicate that the growing conditions affect the elemental composition of yerba mate and the quality of its products.

Keywords: *Ilex paraguariensis*; geographical origin; nutrients; mate tea; soil parent material; multivariate approach.

3.3 INTRODUCTION

Yerba mate (*Ilex paraguariensis* A. St.Hill.) is an endemic tree species from the southern region of South America (Reitz et al., 1988). It is grown in specific regions of Brazil, Argentina and Paraguay, mainly for beverage production from leaves and branches (Valduga et al., 2019). This species has significant economic, social, and environmental contributions in these regions, and it is a supplementary income source for small farms. Such an importance is due to yerba mate productive longevity and because there is the possibility of cultivating it in agroforestry system (Marques, 2014).

Studies have attributed to yerba mate leaves several therapeutic properties (Bracesco et al., 2011; Valduga et al., 2019), which habilitated it to take part in drug and food composition. In addition, studies on yerba mate have demonstrated great nutritional potential due to its ability to accumulate micronutrients, especially manganese (Mn), iron (Fe), zinc (Zn), copper (Cu) and nickel (Ni) (Reissmann et al., 1999; Oliva et al., 2014; Barbosa et al., 2015; Barbosa et al., 2020; Magri et al., 2020).

The literature has pointed to a huge variation in nutrient levels in yerba mate leaves that can be associated with soil chemical characteristics (Toppel et al., 2018; Frigo et al., 2020), soil parent material (Motta et al., 2020), and fertilization (Santin et al., 2013a; Barbosa et al., 2018). Climate and parent material have provided contrasting effects on soil properties (Fontes, 2012; Rabel et al., 2018; Barbosa et al., 2021). On the other hand, climatic characteristics have directly affected plant physiology, especially photosynthesis and transpiration (Taiz et al. 2017). In this sense, temperature can alter the absorption of nutrients (Onwuka and Mang, 2018), with consequences on the elemental composition of plant leaves and consequently on the products made of them. Effects of edaphoclimatic characteristics on elemental composition of some plant species have been reported in South America (Miranda et al., 2013; Barbosa et al., 2019) but similar studies about yerba mate are scarce.

Although cultivated for decades, the knowledge about the elemental composition of yerba mate is restricted to specific regions or greenhouse trials. The elemental composition of yerba mate leaves may be rather affected by edaphoclimatic characteristics than by the management system. Based on the knowledge about yerba mate, this work aims to evaluate the influence of edaphoclimatic conditions on the elemental composition of yerba mate leaves.

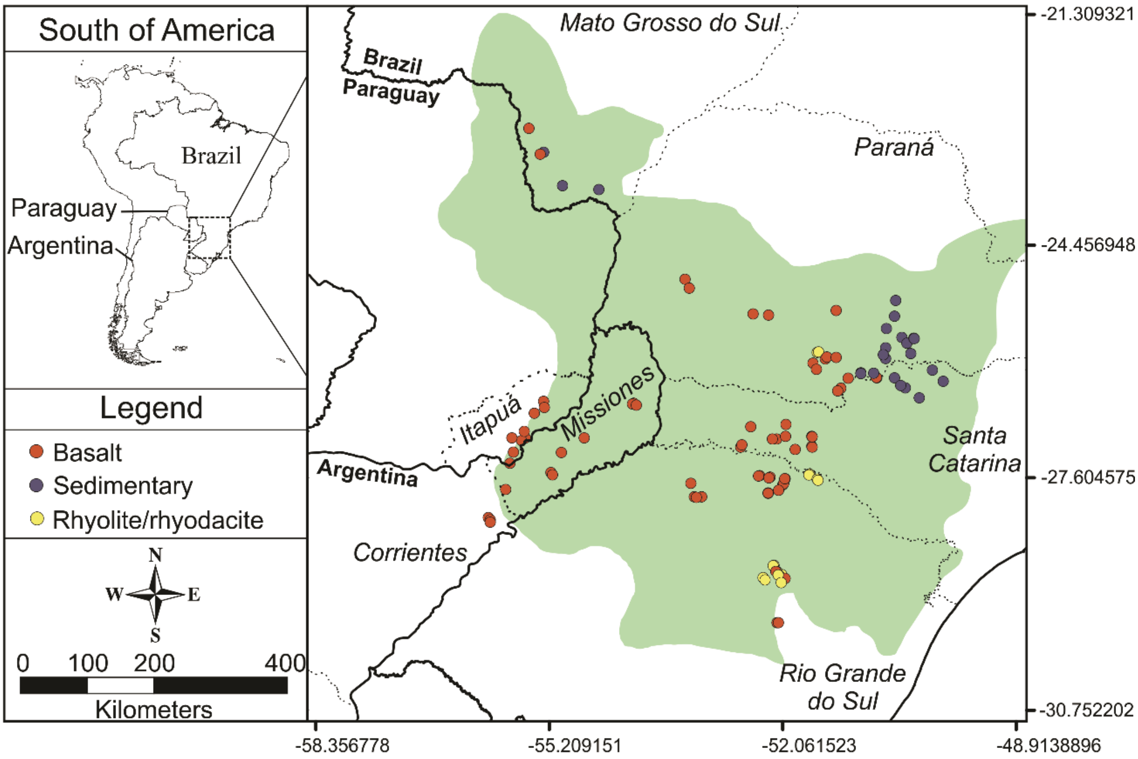
3.4 MATERIALS AND METHODS

3.4.1 STUDY AREA AND SAMPLING

Pairwise samples of yerba mate leaves and soil were collected from 115 farms in 70 locations of South America. Sampling sites were selected to represent the main producing regions of yerba mate in the south of South America (FIGURE 1). Samples were collected in December 2017 and January 2018 from yerba mate cultivated as monocultures (n = 67) and in agroforestry (n = 48). Additional information on sampling sites can be obtained from Magri et al. (2021).

Leaf samples were collected from the upper third of the crown from where fully expanded leaves (mature leaves) were taken from 20 random plants in each plot. Leaves were dried in an oven with forced air circulation at 65° C, quartered, ground in a ball mill, and passed through a 0.25 mm sieve. Soil samples were collected at 0-20 cm depth by using a Dutch auger, composed, homogenized and air dried. Composite soil samples were composed of 20 sub-samples.

FIGURE 1 – STUDY SITES (115 FARMS). THE STUDY REGION COVERS THE MAIN PRODUCING LOCATIONS OF YERBA MATE IN SOUTH AMERICA (HIGHLIGHTED IN GREEN – Oliveira and Rotta, 1985): BRAZILIAN STATES OF *MATO GROSSO DO SUL*, *PARANÁ* AND *SANTA CATARINA*; *ITAPUÁ* STATE OF PARAGUAY; CORRIENTES AND MISSIONES STATES IN ARGENTINA.



SOURCE: The author (2021).

3.4.2 ENVIRONMENTAL PARAMETERS

Data on mean annual temperature and precipitation for the sampling locations were obtained in the World Climate Database (Hijmans et al., 2005) at 30” of spatial resolution. Elevation data were collected in the field by using a Garmim Etrex 10 GPS.

3.4.3 SOIL PROPRIETIES DETERMINATION

Air-dried soil samples were sieved in a 2 mm mesh to determine the following attribute values: pH (0.01M $CaCl_2$ - 1:2.5 ratio); Al^{+3} (extracted with KCl 1 mol L^{-1} and determined by titration); Ca^{+2} and Mg^{+2} (extracted with KCl 1 mol L^{-1} and determined by atomic absorption spectrometry); H + Al (extracted with 5 mol L^{-1} calcium acetate); K^{+} (extracted with Mehlich-I and determined by flame emission spectrometry); P (extracted with Mehlich-I and determined by colorimetry); and organic matter content (Walkley-Black method). In addition, the available concentration of Mn, Fe, Zn, Cu, Ni and Ba were determined after Mehlich-I extraction and quantification in an Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). These procedures followed the methods described in Marques and Motta (2003). Particle size analyzes were done according to the Bouyoucos method using a mixture of sodium hydroxide (4 g L^{-1}) and sodium hexametaphosphate (10 g L^{-1}) as a dispersing solution (Gee and Or, 2002).

Soil samples were ground in a porcelain mortar with a pistil and sieved in a 0.2 mm mesh. Mortar-ground samples were digested according to the EPA 3051A method (Usepa, 1995) in a microwave oven. For this, approximately 400 mg of soil sample was weighed, added to a polypropylene tube containing 9 mL of 65% HNO_3 P.A. (Merck®) and 3 mL of 36-38% HCl. This solution remained at rest for 30 min and then it was submitted to the microwave with a power of 1500-1800 W, a heating ramp of 5.5 min, and a maximum temperature of 175°C for 4.5 minutes. After this procedure, the samples were filtered (0.45 µm filter paper) and the volume was measured to 50 mL, using Milli-Q water. Pseudo-total concentrations of Fe, Al, Si, Mg, Mn, Ca, K, P, S, Cu, Zn, B, Ba, and Ni were determined in an ICP-AES. Pseudo-total quantification was run in duplicate and the mean value of samples was used as the final pseudo-total concentration value.

3.4.4 PLANT ANALYSIS

The digestion of plant material was done in 100 ml polypropylene tubes placed in a microwave oven after the addition of 4 ml of HNO_3 (65%) P.A. (Merck®) and 1 ml of H_2O_2 in 0.2 g of tissue sample. This solution remained at rest for 30 min and subsequently 3 ml of ultra-pure water (Milli-Q) was added to the digested plant material. The polypropylene tubes were subjected to 1,300 W in the microwave oven with a heating ramp of 20 min, remaining at 180º C for 15 min. Finally, the aliquots were filtered through a 0.45 µm paper filter and the volume was measured to 50 ml using ultra-pure water. Elemental quantification was performed in an ICP-AES in duplicate and the mean value of samples was used as the final pseudo-total concentration value.

3.4.5 VALIDATION OF ELEMENTARY DETERMINATION

Analytical results and percentage of recovery obtained from soil and plant tissue certified reference material (CRM) are in TABLES S1 and TABLE S2 respectively. The The recovey of soil Fe, Mn, P, Cu, and Zn approached 100% of CRM. Nickel and B respectively reached 129% and 147% of CRM, while the other elements showed recovery rates lower 65% of CRM. It is important to consider that samples for CRM are totally digested in hydrofluoric. In the plant tissue, only Ni and Fe were recovered below 80% rate, with respectively 69% and 76%. The other elements approached 100% of CRM.

3.4.6 SATISTICAL ANALYSIS

Data were tested for normality of residues by the Shapiro-Wilk test, undergoing Box-Cox transformation when normality was not achieved. Samples were categorized according to the material source like soil type and sampling location. Then, elemental concentration of yerba mate leaves was subjected to ANOVA and in case significant F values, similar ones were grouped by the Scott-Knott test ($p < 0.05$). Leaf elemental concentrations and analytical results of soil samples (Mehlich-I and pseudo-total concentrations) were compared according to the cultivation system (agroforestry vs monoculture) by using T-test. From the correlation matrix of normalized and standardized data (mean= zero, and variance= one), it was preformed Canonical Correlation Analyzes (CCA). The CCA was considered significant when at least two canonical pairs were significant ($p < 0.05$). This analysis was initially performed for the entire population, but due to inconsistent results and non-validation of the canonical pairs, it was explored individually for each source material. Statistical tests were run in R software, version 4.0.3 (R Core Team, 2020).

3.5 RESULTS

3.5.1 GROWING CONDITIONS AT YERBA MATE FARMS

The percentage of sites under agroforestry system, climate types, and soil texture has varied among regions (TABELA 1). Agroforestry systems predominantly occurred in Brazil, especially in the states of *Santa Catarina* and *Paraná*. There were different climate conditions in the sampling regions, particularly with respect to mean annual temperature, precipitation, and altitude.

TABLE 1 – PERCENTAGE OF AGROFORESTY SITES, CLIMATE FEATURES, AND SOIL GRANULOMETRY (MEAN ± STANDARD DEVIATION), ACCORDING TO SAMPLING LOCATION AND SOIL PARENT MATERIAL IN THE MAIN YERBA MATE PRODUCING REGIONS IN SOUTH AMERICA (N=115).

		Temperatura					**Granulometria**		
Região	**Agr.**	**Média**	**Max.**	**Min.**	**Precipitação**	**Altitude**	**Areia**	**Silte**	**Argila**
	(%)	------------ °C ------------			(mm year^{-1})	(m)	--------------- % ---------------		
Basalto									
AR	8	20±1,0	31±1,3	8±1,1	1712±80	360±175	14±3,9	19±7,2	67±8,5
PY	10	21±0,3	32±0,5	10±0,3	1698±52	270±232	17±31	28±5,1	55±4,3
RS	26	18±0,7	28±1,0	9±0,7	1749±173	598±206	9±11	22±4,2	69±1,3
SC	40	17±0,8	28±0,8	7±1,5	1883±199	824±136	14±12	20±4,9	66±14
PR	57	17±0,7	27±0,8	6±0,7	1750±61	943±152	21±9,0	18±5,2	61±11
MS	67	22±0,2	30±0,1	11±0,1	1536±24	494±13	30±8,6	10±3,5	60±5,0
Riolito/riodacito									
RS	50	17±0,5	27±0,7	8±1,0	1703±59	707±78	14±57	30±11	56±12
PR	50	16±0,7	26±0,9	6±0,9	1764±25	1168±10	10±5,6	19±1,9	71±3,8
Sedimentar									
SC	100	17±0,2	27±0,3	5±0,2	1448±57	843±51	20±14	26±7,7	54±20
PR	60	17±0,4	27±0,4	6±0,6	1515±53	818±30	24±15	20±7,1	56±14
MS	33	22±0,2	31±0,4	11±0,1	1589±18	398±98	44±27	9±5,4	47±22

SOURCE: The author (2021).

LEGEND: RS= *Rio Grande* do *Sul* (N=35); SC= *Santa Catarina* (N=21), PR= *Paraná* (N=31); MS= *Mato Grosso do Sul* (N=6); AR= Argentina (N=12); PY= Paraguay (N=10). Agr.= Percentage of agroforestry system.

TABELA 2 – CHEMICAL PROPRIETIES OF SOILS (MEAN ± STANDARD DEVIATION), ACCORDING TO SAMPLING LOCATION AND SOIL PARENT MATERIAL IN THE MAIN YERBA MATE PRODUCING REGIONS IN SOUTH AMERICA (N=115).

Região	**pH**	**Al^{3+}**	**H + Al**	**m**	**V**	**Ca^{2+}**	**Mg^{2+}**	**K^{+}**	**P**	**OM**
	$CaCl_2$	$cmol_c$ dm^{-3}		----- % -----		------ $cmol_c$ dm^{-3} ------			mg dm^{-3}	g dm^{-3}
					Basalto					
AR	3,9±0,2	2,5±1,1	11,7±2,2	46±27	24±15	3,1±2,7	0,7±0,7	0,18±0,12	1,5±0,9	32±15
PY	5,0±0,7	0,1±0,2	4,9±1,3	2,0±3,0	60±12	6,2±1,9	1,0±0,4	0,44±0,14	3,8±5,2	42±16
RS	4,7±0,7	0,8±1,1	7,8±3,1	16±23	50±24	6,3±4,8	1,9±1,1	0,54±0,43	4,9±4,0	48±13
SC	4,2±0,4	2,1±1,5	11,8±3,4	41±32	30±25	4,4±5,2	1,4±1,5	0,35±0,26	3,4±1,7	83±21
PR	4,0±0,4	2,7±1,9	14,0±4,2	51±33	18±16	1,9±2,0	0,7±1,0	0,25±0,21	1,6±1,3	91±27
MS	4,2±0,2	1,4±0,7	8,5±1,3	36±23	25±13	1,8±0,9	0,9±0,5	0,20±0,08	0,8±0,4	62±10
					Riolito/riodacito					
RS	3,9±0,3	3,7±1,4	14,8±2,9	59±21	17±14	2,1±2,1	0,7±0,6	0,24±0,18	10,3±21,1	64±21
PR	3,8±0,1	4,2±0,7	16,4±1,2	71±16	10±6	1,3±0,9	0,4±0,2	0,10±0,00	1,0±0,2	91±15
					Sedimentar					
SC	3,7±0,2	6,0±2,1	19,0±2,9	75±16	9,0±6,0	0,9±0,4	0,4±0,3	0,37±0,25	11,5±11,7	88±14
PR	3,8±0,3	3,7±2,8	15,3±4,5	63±30	12±12	1,4±1,7	0,5±0,6	0,16±0,06	1,5±1,0	90±16
MS	4,4±0,3	0,2±0,2	3,8±0,6	12±9,0	34±10	1,6±0,7	0,3±0,0	0,10±0,00	2,7±1,2	65±3

SOURCE: The author (2021).

LEGEND: RS= *Rio Grande do Sul* (N=35); SC= *Santa Catarina* (N=21), PR= *Paraná* (N=31); MS= *Mato Grosso do Sul* (N=6); AR= Argentina (N=12); PY= Paraguay (N=10); m: Aluminum saturation. V: Base saturation. OM: Organic matter.

Soil high acidity, great variation in organic matter contents, low concentrations of available P, and exchangeable K have stood out in the sampling soils (TABLE 2). There was a wide variation in the available concentration of micronutrients (Mn, Fe, Zn, Cu and Ni), and Ba among the sampling locations (TABLE 3). Copper concentrations significantly varied according to the parent material, which were high in basaltic soils. Iron (Fe) concentrations in basaltic soils showed lower average values than the other sampled soils. Much higher values of available Mn were also observed in the basaltic soils of *Rio Grande do Sul*, *Santa Catarina* and *Paraná* (Brazil) compared to the other locations.

Elemental pseudo-total concentrations in soils related to the parent materials in study regions (TABLE S3). In general, average concentrations of Mn, Fe, Cu, Ba, and Ni were higher in basaltic soils than in soils formed from rhyolite/rhyodacite or sedimentary rocks. Phosphorus and Zn concentrations were lower in soils formed from sedimentary rocks compared to the other parent materials.

TABLE 3 – MICRONUTRIENTS AND AVAILABLE BA IN SOIL – MEHLICH-I EXTRACTOR (MEAN ± STANDARD DEVIATION), ACCORDING TO SAMPLING LOCATION AND SOIL PARENT MATERIAL IN THE MAIN YERBA MATE PRODUCING REGIONS IN SOUTH AMERICA (N=115).

Region	**Mn**	**Fe**	**Zn**	**Cu**	**Ni**	**Ba**
	------- mg dm^{-3} -------					
			Basalt			
AR	58±38	28±8,0	1,8±0,8	5,4±,2,4	0,4±0,2	1,8±0,3
PY	84±35	41±64	16,0±12,3	8,1±4,3	1,2±0,7	1,7±0,3
RS	267±126	41±13	6,5±10,9	8,6±2,8	0,6±0,6	1,3±0,2
SC	128±121	81±62	7,2±5,8	6,5±2,7	0,7±0,5	1,4±0,2
PR	104±60	89±38	4,9±3,1	10,7±5,6	0,5±0,3	1,9±0,4
MS	51±32	42±14	2,2±0,4	6,4±0,9	0,1±0,0	1,6±0,3
			Rhyolite/rhyodacite			
RS	69±36	125±51	3,1±1,9	2,7±2,4	0,2±0,1	1,3±0,1
PR	22±7,0	150±17	13,2±8,3	1,1±0,3	0,3±0,0	1,8±0,0
			Sedimentary			
SC	25±12	175±76	9,3±3,5	1,8±0,5	1,2±0,6	1,4±0,2
PR	59±92	108±57	3,7±1,6	2,5±1,4	0,4±0,4	2,1±0,6
MS	26±5,0	37±6,0	2,5±0,7	0,9±0,2	0,1±0,0	1,3±0,1

SOURCE: The author (2021).

LEGEND: RS= Rio Grande do Sul (N=35); SC= Santa Catarina (N=21), PR= Paraná (N=31); MS= Mato Grosso do Sul (N=6); AR= Argentina (N=12); PY= Paraguay (N=10).

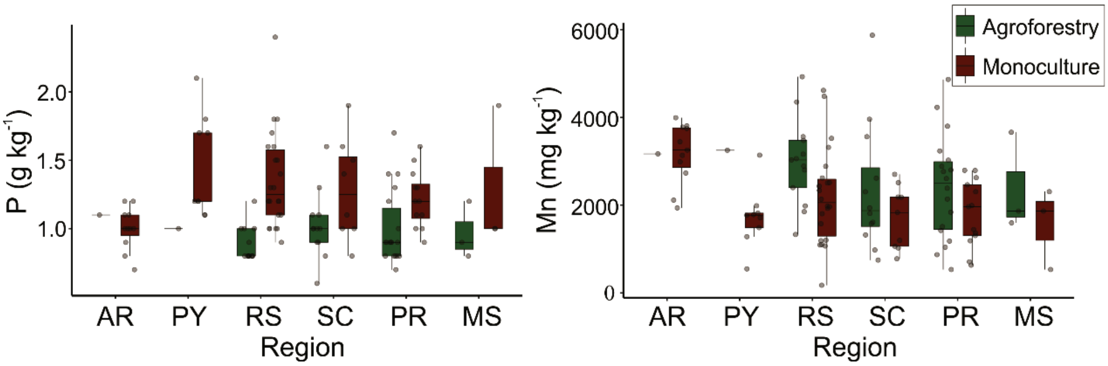
There was also variation in the concentrations of elements among soils originated from the same parent material: basaltic soils significantly differed in the levels of Mn, Ca, K, P, and S. Rrhyolite/rhyodacite soils differed in levels of Mn, P, Cu, Zn, and Ba. Sedimentary soils differed in concentrations of Al, Si, Mg, Mn, K, P, S, Zn, Ba, and Ni, being always lower in the state of *Mato Grosso do Sul* (Brazil).

3.5.2 ELEMENTAL COMPOSITION AT YERBA MATE LEAVES

There was great variation in concentrations of micronutrients, Al, and Ba in yerba mate leaves (TABLE 4), that ranged low to extreme values. Samples from Paraguay and Rio Grande do Sul state (Brazil) presented the highest values for practically all the elements analyzed and statistical tests confirmed such results. Lower elemental concentrations in leaves were usually from plants grown on sedimentary soils.

Leaf concentrations of P and Mn showed significant differences between plants cultivated in agroforestry systems and as monoculture, regardless of the soil parent material. Samples of yerba mate leaves from the agroforestry system showed lower concentrations of P (p <0.05) and higher concentrations of Mn (p <0.05) compared plants from monocultures (FIGURE 2).

FIGURE 2 – PHOSPHORUS AND MN CONCENTRATIONS IN YERBA MATE LEAVES ACCORDING TO PRODUCTION SYSTEM AND SAMPLING REGION OF THE MAIN YERBA MATE PRODUCING LOCATIONS IN SOUTH AMERICA (N = 115). P AND MN CONCENTRATIONS PRESENTED DIFFERENCES ACCORDING TO PRODUCTION SYSTEM.



SOURCE: The author (2021).

LEGEND: RS= *Rio Grande do Sul* (N=35); SC= *Santa Catarina* (N=21), PR= *Paraná* (N=31); MS= *Mato Grosso do Sul* (N=6); AR= Argentina (N=12); PY= Paraguay (N=10).

TABLE 4 – ELEMENTAL COMPOSITION OF YERBA MATE LEAVES (MEAN ± STANDARD DEVIATION), ACCORDING TO SAMPLING LOCATION AND SOIL PARENT MATERIAL IN THE MAIN YERBA MATE PRODUCING REGIONS IN SOUTH AMERICA (N=115).

Region	**C**	**N**	**K**	**Ca**	**Mg**	**S**	**P**
	--------- % ---------		------------------------------------ g kg^{-1} ------------------------------------				
				Basalt			
AR	45,9±1,38	2,49±0,34	11,8±1,4^{b}	6,52±1,75^{b}	5,92±1,73^{b}	1,28±0,16	1,01±0,15
PY	45,8±1,99	2,2±0,35	15,5±1,8^{a}	8,61±1,29^{a}	6,85±1,16^{a}	1,38±0,21	1,41±0,38
RS	45,9±1,73	2,47±0,36	15,0±2,2^{a}	7,36±1,82^{a}	7,09±1,76^{a}	1,41±0,20	1,15±0,25
SC	45,5±2,56	2,62±0,59	15,0±2,5^{a}	7,24±2,31^{a}	6,37±1,89^{a}	1,5,0±0,18	1,39±0,97
PR	47,1±1,90	2,69±0,3	14,8±2,9^{a}	6,32±1,60^{b}	5,49±1,72^{b}	1,37±0,20	1,09±0,26
MS	46,7±2,08	2,47±0,25	12,6±4,6^{b}	5,93±1,08^{b}	7,63±2,25^{a}	1,2,0±0,20	0,90±0,10
				Rhyolite/rhyodacite			
RS	46,2±1,59	2,36±0,44	15,5±2,7^{a}	7,39±1,76^{a}	6,59±1,69^{a}	1,37±0,17	1,21±0,46
PR	46,5±2,12	2,45±0,35	12,1±0,1^{b}	6,00±2,69^{b}	5,75±2,62^{b}	1,20±0,14	1,00±0,28
				Sedimentary			
SC	47,5±2,07	2,67±0,35	17,4±1,22^{a}	5,70±1,35^{b}	4,40±1,16^{b}	1,43±0,15	1,08±0,31
PR	47,2±1,70	2,72±0,46	15,3±1,96^{a}	6,03±1,09^{b}	5,50±1,51^{b}	1,37±0,26	1,25±0,64
MS	46,7±0,58	2,23±0,67	12,3±2,80^{b}	6,17±0,45^{b}	6,60±1,30^{a}	1,27±0,38	1,37±0,47
p-value	0,08	0,2	<0,01	0,01	0,02	0,06	0,41

Region	**Mn**	**Al**	**Fe**	**Zn**	**Ba**	**B**	**Cu**	**Ni**
	-- mg kg^{-1} --							
				Basalt				
AR	3175±655^{a}	524±88^{a}	129±46^{a}	53±28^{b}	68±26^{a}	63±15^{b}	10,7±3,6^{a}	4,7±1,3^{a}
PY	1878±804^{b}	454±146^{a}	182±58^{a}	191±154^{a}	65±22^{a}	105±27^{a}	8,8±1,0^{b}	5,1±2,8^{a}
RS	2616±968^{a}	403±110^{b}	182±113^{a}	102±116^{a}	56±23^{a}	51±23^{c}	10,4±2,9^{a}	3,9±1,9^{a}
SC	1949±964^{b}	502±216^{a}	201±130^{a}	86±75^{a}	64±28^{a}	35±12^{d}	10,3±3,3^{a}	3,5±2,1^{a}
PR	2535±1134^{a}	353±88^{b}	100±32^{b}	57±42^{b}	49±21^{b}	35±9^{d}	11,4±2,3^{a}	4,0±1,3^{a}
MS	2611±936^{a}	483±83^{a}	183±46^{a}	17±3^{c}	58±20^{a}	68±8^{b}	9,0±1,3^{b}	3,7±2,2^{a}
				Rhyolite/rhyodacite				
RS	2378±1253^{a}	399±130^{b}	143±46^{a}	45±31^{b}	61±22^{a}	37±13^{d}	9,3±1,6^{b}	2,4±1,5^{b}
PR	1514±460^{b}	276±77^{b}	72±6^{c}	47±43^{b}	39±25^{b}	29±3^{e}	9,4±2,0^{b}	1,4±0,7^{b}
				Sedimentary				
SC	2423±1780^{a}	383±117^{b}	122±109^{b}	46±37,0^{b}	35±6,26^{b}	23±4,97^{e}	11,0±3,5^{a}	3,1±1,1^{a}
PR	1880±959^{b}	350±105^{b}	82±17^{c}	45,3±43,2^{b}	44±17,5^{b}	28±11,3^{e}	12,0±3,4^{a}	3,9±2,2^{a}
MS	1332±705^{b}	400±128^{b}	145±7^{a}	29,3±19,7^{c}	37±13,0^{b}	34±4,9^{d}	8,3±1,5^{b}	1,4±0,3^{b}
p-value	0,03	0,01	p<0,01	p<0,01	p<0,01	p<0,01	0,05	p<0,01

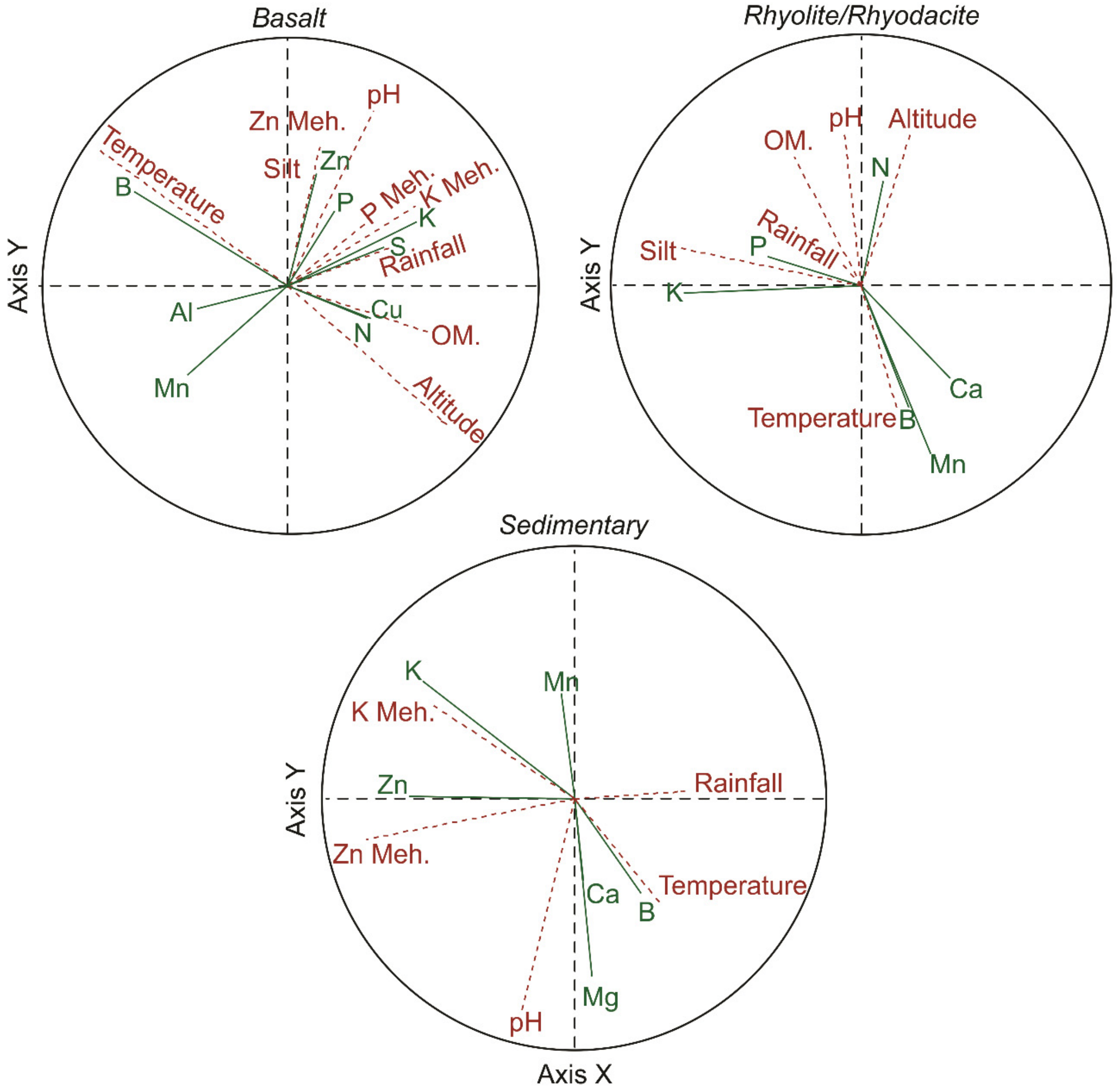
SOURCE: The author (2021).

LEGEND: RS= *Rio Grande do Sul* (N=35); SC= *Santa Catarina* (N=21), PR= *Paraná* (N=31); MS= *Mato Grosso do Sul* (N=6); AR= Argentina (N=12); PY= Paraguay (N=10). Means of the same letter in columns do not significantly differ by ANOVA followed Scott-Knott test (p<0.05).

3.5.3 RELATIONSHIP AMONG ELEMENTAL CONCENTRATION IN YERBA MATE LEAVES, CLIMATE, AND SOIL

There was no significant relationship between elemental composition of yerba mate leaves and edaphoclimatic properties when all samples and cultivation systems were analyzed at once in a multivariate approach. It was found different relationships when data were separately analyzed for each soil type and parent material (FIGURE 3).

FIGURE 3 – CANONICAL CORRESPONDENCE ANALYSIS (CCA) OF FOLIAR ELEMENTAL COMPOSITION OF YERBA MATE LEAVES, SOIL, CLIMATE, AND PARENT MATERIAL FROM THE MAIN YERBA MATE PRODUCING REGIONS IN SOUTH AMERICA.



SOURCE: The author (2021).

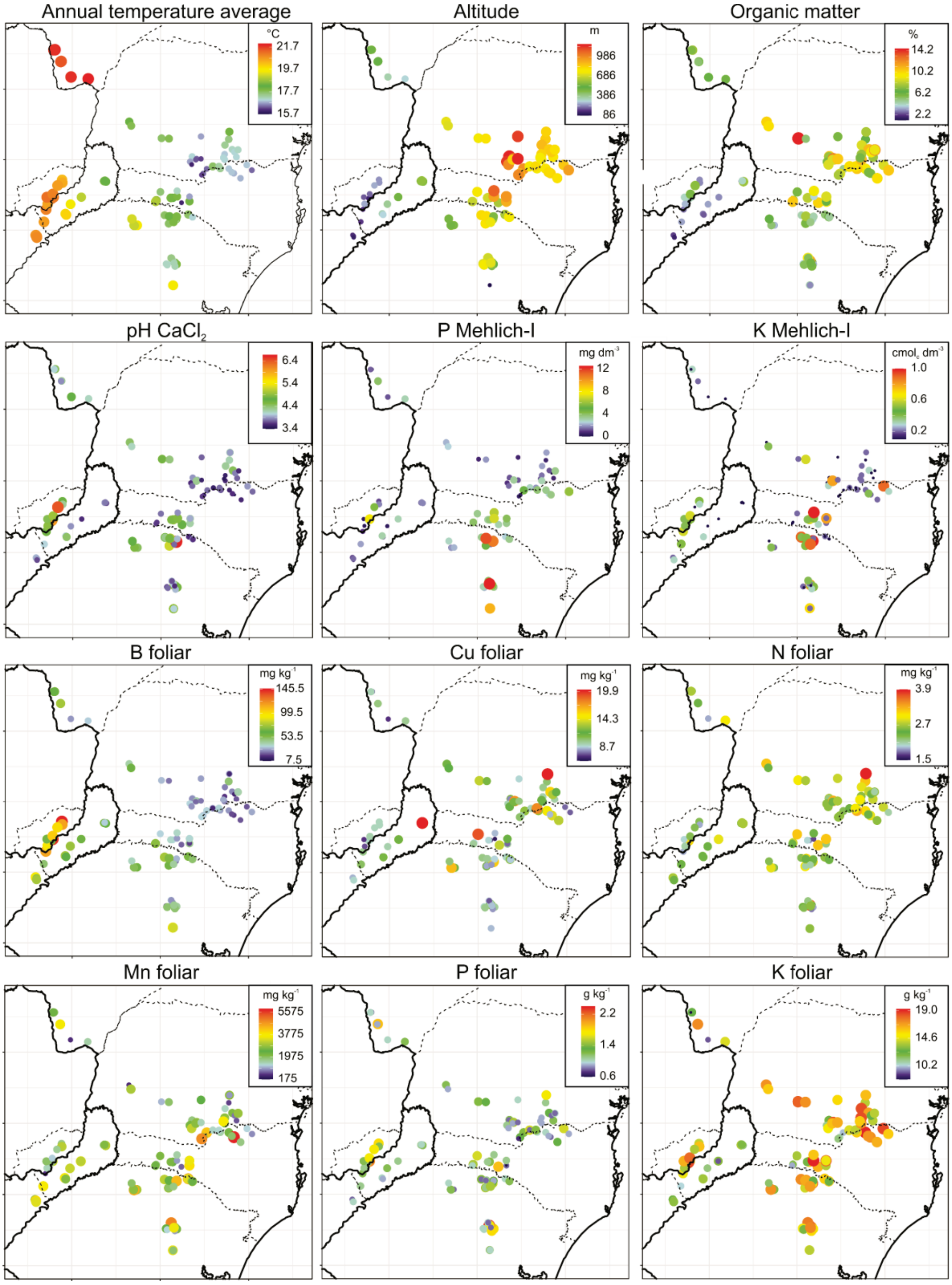
LEGEND: OM.= Organic matter; MEH.= Mehlich-I extraction.

Canonical Correlation Analysis - CCA (FIGURE 3) of yerba mate leaves grown on basaltic soils revealed an association between leaf concentrations of Cu and N with organic matter and altitude. These same variables (Cu and N) negatively correlated with mean annual temperature, which showed association with B concentrations (FIGURE 4). There was also negative correlation between soil pH and levels of Mn and Al in plant leaves and between the amount of silt and Zn and P contents in leaves. Soil available P and Zn and exchangeable K positively correlated with the respective contents of these elements in plant leaves.

This multivariate approach (CCA) for plants grown on soils originated from rhyolite/rhyodacite (FIGURE 3) revealed an association between K and P in plant tissue with soil silt content. There was also a negative correlation between Mn in leaves and soil pH and association between B and Ca with mean annual temperature, between leaf N with soil organic matter and altitude, as previously mentioned for basaltic soils.

For plants grown on soils originated from sedimentary rocks, the CCA showed relationship between soil exchangeable K and available Zn with the respective elements in plan tissue. Concentrations of Ca and B in leaves positively correlated with temperature, and Mn negatively correlated with soil pH.

FIGURE 4 – CLIMATE, SOIL PROPERTIES, AND FOLIAR ELEMENTAL CONCENTRATIONS OF MAIN INTERACTIONS IN THE MAIN PLACES OF YERBA MATE PRODUCTION IN SOUTH AMERICA.



SOURCE: The author (2021).

3.6 DISCUSSION

The extractivism of native yerba mate are a historic activity in the producing states of Brazil (Marques, 2014), which tradition has been maintained mainly in the state of *Paraná*. This explains the high occurrence of yerba mate in agroforestry systems in Paraná state. It has been registered only one yerba mate site under agroforestry system in Paraguay and Argentina each, where prevails the monoculture of this species. Despite the high acidity of majority sampled soils and the presence of Al^{3+}, these characteristics has not represented a problem for yerba mate growth as the species is tolerant to very acidic soils (Reissmann et al., 1999). This fact has been observed by Benedetti et al (2017), who have found a positive effect of Al^{3+} on root growth of yerba mate plants. This result corroborates Haridasan (2008), who has demonstrated the adaption of native plants to acidic soils and Al^{+3} in the tropics. There are restrictions on lime application to soils under yerba mate cultivation and such practice has been only indicated for Ca and Mg replacement and not for amending soil acidity (Pauletti and Motta, 2017; Silva et al., 2016).

Most of the sampled sites have shown either low levels of exchangeable K (22% or < 0.12 $cmol_c$ dm^{-3}) or medium levels of it (33% or 0.13 – 0.21 $cmol_c$ dm^{-3}) (Pauletti and Motta, 2017). Levels of available P were very low 43% of sites (< 2 mg dm^{-3}), low in 36% of sites (2 - 4 mg dm^{-3}), medium in 10% of sites (4 - 7 mg dm^{-3}) (Pauletti and Motta, 2017), and very high in only 11% of sites (> 7 mg dm^{-3}) (FIGURE 4). Although low values of available P are a natural feature of these soils, successive harvests of yerba mate leaves and branches over the years may have contributed to it (Motta et al., 2020). Nutritional depletion that reduced soil exchangeable cations due to successive cultivation has been diagnosed in soils under forests of *Pinus taeda* in southern Brazil (Gatiboni et al., 2020). Thus, our results indicate that there is a general shortage of P in soils of South America under yerba mate production due to the absence of P fertilization, which is still poorly adopted by producers and certainly result in losses of productivity.

Levels of P and Mn in leaves have depended on the cultivation system (FIGURE 2) despite the similar concentration values of these nutrients in soils (FIGURE S1). Levels of Mn in leaves have been higher in yerba mate in agroforestry system than in monocultures. However, plants cultivated as monoculture have shown higher levels of P than plants in agroforestry system. Yerba mate roots establish association with mycorrhizae and there is greater colonization when the species grows under natural conditions (Bergottini et al., 2017; Silvana et al., 2020). In impoverished P environments, mycorrhizae releases exudates that acidify the rhizosphere as a mechanism for P acquisition (Lambers et al., 2020). As a result, Mn accumulation can occur as a side effect due to the great rhizosphere acidification as evidenced for the Mn hyper-accumulating species *Phytolacca americana* (DeGroote et al., 2018). Since yerba mate is also a Mn hyper-accumulating species (Magri et al., 2020), the same process may apply to it. The CCA for basaltic soils (FIGURE 3) has indicated the existence of an inverse relationship between the contents of Mn in leaves and soil available P. Barbosa et al. (2018) have found decreases in Mn levels in leaves of yerba mate in response to P fertilization in a greenhouse experiment. Thus, the higher levels of Mn in plants from agroforestry may be a consequence of low P values in soils and mycorrhizal activity as well. In addition to possible mycorrhizal activity, the higher Mn content in yerba mate leaves in plants from agroforestry may relate to air temperature and humidity with this system in relation to crops under full sun. Forests keep soil moisture higher and more constant, and mean temperature is milder than in plantations under full sun. These conditions contribute to the greater availability of Mn (Lambers et al., 2020).

A common effect in this study has been the negative relationship between soil pH and leaf Mn concentrations (FIGURE 3). Values of pH is one the main factors that control Mn in soils (Turner and Blackwell 2013; Millaleo et al., 2010). Our results have corroborated greenhouse experiments with seedlings of yerba mate (Santin et al., 2013b; Magri et al., 2020) that have shown that Mn is the most affected element in yerba mate leaves after increasing soil pH.

Another common effect in the study locations was the correlation between concentrations of Ca and B in leaves and mean annual temperature (FIGURE 3). This effect has been so incident that it has become contrasting when observing these results for each study location (FIGURE 4). Factors that affect plant transpiration such as high air temperatures directly affect the absorption of Ca and B due to the predominant mass flow mechanism of ion-root contact (Broadley et al., 2012). It explains the association of Ca and B with mean annual temperature, since plants subjected to high temperatures absorb and release more water and consequently have a closer contact with Ca and B. However, concentrations of Ca in yerba mate leaves have also been influenced by soil exchangeable Ca (Santin et al., 2013b).

Calcium concentrations in yerba mate leaves have seemed partially dependent on climate, while B in leaves of yerba mate seems to be rather dependent on climate than on soil characteristics.

Positive correlations of soil organic matter and altitude with leaf N and Cu have been significant on rhyodacite and basaltic soils (Figure 3), since these elements are closely associated with the soil organic matter (Plante and Parton, 2007). Altitude and mean annual temperature are inversely related (AVRDC, 1990) and they are factors that influence organic matter stabilization in soils (Bayer et al., 2006) and consequently stocks of N and Cu. Thus, this result reveals the importance of organic matter as nutrient source for yerba mate plants.

Concentrations of available P, Zn, and exchangeable K have been respectively associated with these element concentrations in the leaves from plants grown on basaltic soils. Similar association has occurred for K and Zn in plants grown on sedimentary soils. Mehlich-I extractor has been adopted by the laboratories located in the main yerba mate producing regions of Brazil (Pauletti and Motta, 2017; Silva et al., 2016); and our results have shown its efficiency for P and K extraction in areas of basaltic soils, but it may not be as efficient K extraction in areas of rhyolite/rhyodacite.

There has been also an association between silt content and leaf concentrations of P and K in areas of basaltic and rhyolite/rhyodacite soils, which may indicate that these elements may be being absorbed from less accessible fractions. There may be mineral reserves of P and K in these soils that are accessible to yerba mate plants because of the low measured concentrations of available P and exchangeable K. As previously discussed, P shortage in soils trigger mycorrhizae exudations and allow the acquisition of elements from a less accessible sources (Lambers et al., 2020). Such results also indicates a high capacity of yerba mate plants for extracting these elements from soils. Variations in the elemental composition of yerba mate in response to site characteristics must affect the quality of the products derived from its leaves (Magri et al., 2021; Ulbrich et al., 2021).

3.7 CONCLUSION

The significant variations observed in yerba mate plants in response to climate types, soil characteristics, and cultivation systems revealed an interesting species plasticity, which makes possible to cultivate it under different environmental conditions. Concentrations of micronutrients, Al, and Ba in yerba mate leaves have been usually lower in plants grown on soils originated from sedimentary rock parent material. The highest elemental values was measured in plants grown on soils originated from igneous rocks, especially in Paraguay and *Rio Grande do Sul* state (Brazil). Samples of yerba mate leaves from agroforestry systems had lower concentrations of P and higher concentrations of Mn compared to plants cultivated as monoculture.

There was a negative correlation between leaf Mn and soil pH and a positive correlation between B and Ca leaf with mean annual temperature. Concentrations of leaf N in plants grown on soils originated from igneous rocks correlated with soil organic matter content and altitude. Mehlich-I extractor has shown to be efficient for extracting P and K from basaltic soils and K from soils originated from rhyolite/rhyodacite.

Cultivation system, altitude and temperature have directly affected the elemental composition yerba mate leaves, especially P, Mn, B, and N. Growth conditions under which yerba mate was cultivated had implications on the elemental composition of leaves.

3.8 REFERENCES

AVRDC. (1990). Vegetable Production Training Manual. Tainan: Asian Vegetable Research and Development Center. 447p.

Barbosa, J. Z., dos Santos Domingues, C. R., Poggere, G. C., Motta, A. C. V., Dos Reis, A. R., de Moraes, M. F., & Prior, S. A. (2019). Elemental composition and nutritional value of Araucaria angustifolia seeds from subtropical Brazil. *Journal of food science and technology*, 56(2), 1073-1077.

Barbosa, J. Z., Motta, A. C., Consalter, R., Poggere, G. C., Santin, D., & Wendling, I. (2018). Plant growth, nutrients and potentially toxic elements in leaves of yerba mate clones in response to phosphorus in acid soils. *Anais da Academia Brasileira de Ciências*, 90(1), 557-571.

Barbosa, J. Z., Motta, A. C. V., dos Reis, A. R., Corrêa, R. S., & Prior, S. A. (2020). Spatial distribution of structural elements in leaves of *Ilex paraguariensis*: physiological and ecological implications. *Trees*, 34(1), 101-110.

Barbosa, J. Z., Zambon, L. M., Motta, A. C. V. & Wendling, I. (2015). Composition, hot-water solubility of elements and nutritional value of fruits and leaves of yerba-mate. *Ciência e Agrotecnologia,* 39(6), 593–603.

Bayer, C., Lovato, T., Dieckow, J., Zanatta, J. A., & Mielniczuk, J. (2006). A method for estimating coefficients of soil organic matter dynamics based on long-term experiments. *Soil and Tillage Research*, 91(1-2), 217-226.

Bergottini, V. M., Hervé, V., Sosa, D. A., Otegui, M. B., Zapata, P. D., & Junier, P. (2017). Exploring the diversity of the root-associated microbiome of *Ilex paraguariensis* St. Hil.(Yerba Mate). *Applied Soil Ecology*, 109, 23-31.

Bracesco, N., Sanchez, A. G., Contreras, V., Menini, T., & Gugliucci, A. (2011). Recent advances on *Ilex paraguariensis* research: minireview. *Journal of ethnopharmacology*, 136(3), 378-384.

Broadley, M., Brown, P., Cakmak, I., Rengel, Z., & Zhao, F. (2012). Function of nutrients: micronutrients. *In Marschner's mineral nutrition of higher plants* (pp. 191-248). Academic Press.

DeGroote, K. V., McCartha, G. L., & Pollard, A. J. (2018). Interactions of the manganese hyperaccumulator *Phytolacca americana* L. with soil pH and phosphate. *Ecological research*, 33(4), 749-755.

SOURCEs, M. P. F. (2012). Intemperismo de rochas e minerais*. Pedologia-Fundamentos.* Viçosa, MG: Sociedade Brasileira de Ciência do Solo, 171-205.

Frigo, C., Magri, E., Barbosa, J. Z., Sarteretto, L. M., Araujo, E. M., Prior, S. A., & Motta, A. C. V. (2020). Influence of roadways on heavy metal content in soils and yerba mate tissue in southern Brazil. *Management of Environmental Quality: An International Journal,* 31 No. 6, pp. 1477-1495. https://doi.org/10.1108/MEQ-10-2019-0219.

Gatiboni, L. C., Da Silva, W. C., Mumbach, G. L., Schmitt, D. E., Iochims, D. A., Stahl, J., & Vargas, C. O. (2020). Use of exchangeable and nonexchangeable forms of calcium, magnesium, and potassium in soils without fertilization after successive cultivations with *Pinus taeda* in southern Brazil. *Journal of Soils and Sediments*, 20(2), 665-674.

Gee, G.W. & Or, D. (2002). 2.4 Particle-size analysis. *Methods of soil analysis: Part 4 physical methods*, 5, 255-293.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology: A Journal of the Royal Meteorological Society*. 25, 1965–1978. https://doi.org/10.1002/joc.1276.

Lambers, H., Wright, I. J., Pereira, C. G., Bellingham, P. J., Bentley, L. P., Boonman, A., Cernusak, L. A., Foulds, W., Gleason, S. M., Gray, E. F., Hayes, P. E., Kooyman, R. M., Malhi, Y., Richardson, S. J., Shane, M. W., Staudinger, C., Stock, W. D., Swarts, N. D., Turner, B. L., Turner, J., Veneklaas, E., Wasaki, J., Westoby, M. & Xu, Y. (2020). Leaf manganese concentrations as a tool to assess belowground plant functioning in phosphorus-impoverished environments. *Plant and Soil*, 1-19.

Magri, E., Gugelmin, E. K., Grabarski, F. A. P., Barbosa, J. Z., Auler, A. C., Wendling, I., Prior, S. A., Valduga, A. T., Motta, A. C. V., 2020. Manganese hyperaccumulation capacity of *Ilex paraguariensis* A. St. Hil. and occurrence of interveinal chlorosis induced by transient toxicity. Ecotoxicology and Environmental Safety, 203, 111010.

Magri, E., Valduga, A. T., Gonçalves, I. L., Barbosa, J. Z., de Oliveira Rabel, D., Menezes, I. M. N. R., Nascimento, P. A., Oliveira, A., Corrêa, R. S. & Motta, A. C. V. (2021). Cadmium and lead concentrations in yerba mate leaves from agroforestry and plantation systems: An international survey in South America. *Journal of Food Composition and Analysis*, 96, 103702.

Marques, A. C. (2014). As paisagens do mate e a conservação socioambiental: um estudo junto aos agricultores familiares do planalto norte catarinense (434 p. (Tese, curso de Doutorado em Meio Ambiente e Desenvolvimento da Universidade Federal do Paraná)).

Marques, R. & Motta, A. C. V. (2003). Análise química do solo para fins de fertilidade. In: Lima, M.R.de. (Ed.), *Manual de diagnóstico da fertilidade e manejo dos solos agrícolas, 2nd ed.* Departamento de Solos e Engenharia Agrícola, Curitiba, pp. 81–102.

Millaleo, R., Reyes-Diaz, M., Ivanov, A. G., Mora, M. L. & Alberdi, M. (2010). Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition*, 10 (4), 470–481.

Miranda, M.; Vega-Gálvez, A.; Martínez, E. A.; López, J.; Marín, R.; Aranda, M. & Fuentes, F. Influence of contrasting environments on seed composition of two quinoa genotypes: nutritional and functional properties. *Chilean Journal of Agricultural Research*, 73(2):108-116, 2013.

Motta, A. C. V., Barbosa, J. Z., Magri, E., Pedreira, G. Q., Santin, D., Prior, S. A., Consalter, R., Young, S. D., Broadley, M. R. & Benedetti, E. L. (2020). Elemental composition of yerba mate (*Ilex paraguariensis* A. St.-Hil.) under low input systems of southern Brazil. *Science of the Total Environment,* 736, 139637.

Onwuka, B. & Mang, B. (2018). Effects of soil temperature on some soil properties and plant growth. *Scholarly Journal of Agricultural Science*, Res, 8, 34-37.

Pauletti, V. & Motta, A. C. V. (2017). (Coord.). Manual de Adubação e Calagem: Para o Estado do Paraná. *Curitiba: Sociedade Brasileira de Ciência do Solo - Núcleo Estadual do Paraná*. 482 p.

Penn, C. J. & Camberato, J. J., 2019. A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture*, 9(6), 120.

Plante, A. F. & Parton, W. J. (2007). The dynamics of soil organic matter and nutrient cycling. In *Soil microbiology, ecology and biochemistry* (pp. 433-467). Academic Press.

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reissmann, C. B., Radomski, M. I., Quadros, R. M. B. D., 1999. Chemical Reissmann, C. B., Radomski, M. I. & Quadros, R. M. B. (1999). Chemical composition of *Ilex paraguariensis* St. Hil. under different management conditions in seven localities of Paraná State. *Brazilian Archives of Biology and Technology,* 42(2), 187–194.

Reitz, R., Klein, R. M., & Rus, A. (1988). *Projeto madeira do Rio Grande do Sul: termo de referencia: estudo da materia prima: levantamento das especies florestais nativas com possibilidade de incremento e desenvolvimento*. Sudesul.

Santin, D., Benedetti, E. L., Bastos, M. C., Kaseker, J. F., Reissmann, C. B., Brondani, G. E. & de Barros, N. F. (2013a). Crescimento e nutrição de erva-mate influenciados pela adubação nitrogenada, fosfatada e potássica. *Ciência Florestal*, 23(2), 363-375.

Santin, D., Benedetti, E. L., Kaseker, J. F., Bastos, M. C., Reissmann, C. B., Wendling, I. & Barros, N. F. D. (2013b). Nutrição e crescimento da erva-mate submetida à calagem. *Ciência Florestal*, 23(1), 55-66.

Silva, L. D. S., Gatiboni, L., Anghinoni, I. & Souza, R. (2016). Manual de calagem e adubação: Para os estados do Rio Grande do Sul e Santa Catarina. *Sociedade Brasileira de Ciência do Solo - Núcleo Regional Sul*, Comissão de Química e Fertilidade do Solo. Porto Alegre, 376 p.

Silvana, V. M., Carlos, F. J., Lucía, A. C., Natalia, A. & Marta, C. (2020). Colonization dynamics of arbuscular mycorrhizal fungi (AMF) in *Ilex paraguariensis* crops: Seasonality and influence of management practices. *Journal of King Saud University – Science*, 32(1), 183-188.

Taiz, L., Zeiger, E., Møller, I. M. & Murphy, A. (2017). *Fisiologia e desenvolvimento vegetal.* Artmed Editora.

Toppel, F. V., Junior, A. M., Motta, A. C. V., Frigo, C., Magri, E. & Barbosa, J. Z. (2018). Soil chemical attributes and their influence on elemental composition of yerba mate leaves. *Floresta*, 48(3), 425-434.

Turner B. L. & Blackwell M. S. A. (2013). Isolating the influence of pH on the amounts and forms of soil organic phosphorus. *European Journal of Soil Science*, 64:249–259. https://doi.org/10.1111/ejss.12026

Usepa, E. (1995). Method 3051: Microwave assisted acid digestion of sediments, sludges, soils, and oils. *Test methods for evaluating solid waste*, pp 1-30.

Valduga, A. T., Gonçalves, I. L., Magri, E. & Finzer, J. R. D. (2019). Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. *Food Research International*. Int. 120, 478–503.

3.9 SUPPLEMENTAY MATERIAL

TABLE S1 – ANALYSIS OF CERTIFIED REFERENCE MATERIAL (CRM) 2710A – MONTANA I SOIL.

Element	**Value certified**	**Value measured**	**Recovery**
	------------ mg kg^{-1}	------------	%
Al	59500	24798	42
B	20	29	147
Ba	792	511	65
Ca	9640	2321	24
Cu	3420	3286	96
Fe	43200	44436	103
K	21700	6599	30
Mg	7340	4201	57
Mn	2140	2099	98
Ni	8	10	129
P	1050	1009	96
S	-	-	-
Si	311000	2776	1
Zn	4180	4306	103

SOURCE: The author (2021).

TABLE S2 – ANALYSIS OF CERTIFIED REFERENCE MATERIAL – CRM (GBW–10016 *Camellia sinensis*).

Element	**Value certified**	**Value measured**	**Recovery**
	------------ mg kg^{-1}	------------	%
Al	-	837	-
B	-	10	-
Ba	41	35	86
Ca	12100	11005	91
Cu	24	22	94
Fe	322	246	76
K	15500	16110	104
Mg	2200	1885	86
Mn	1170	1260	108
Ni	5,4	3,7	69
P	2800	2645	94
S	4200	3928	94
Zn	35	31	89

SOURCE: The author (2021).

TABLE S3 – PSEUDO-TOTAL ELEMENTAL CONCENTRATIONS OF TOPSOILS (MEAN ± STANDARD DEVIATION) COLLECTED FROM FARMS LOCATED IN THE MAIN YERBA MATE PRODUCING REGIONS IN SOUTH AMERICA (N=115), ACCORDING TO SAMPLING REGIONS AND SOIL PARENT MATERIAL.

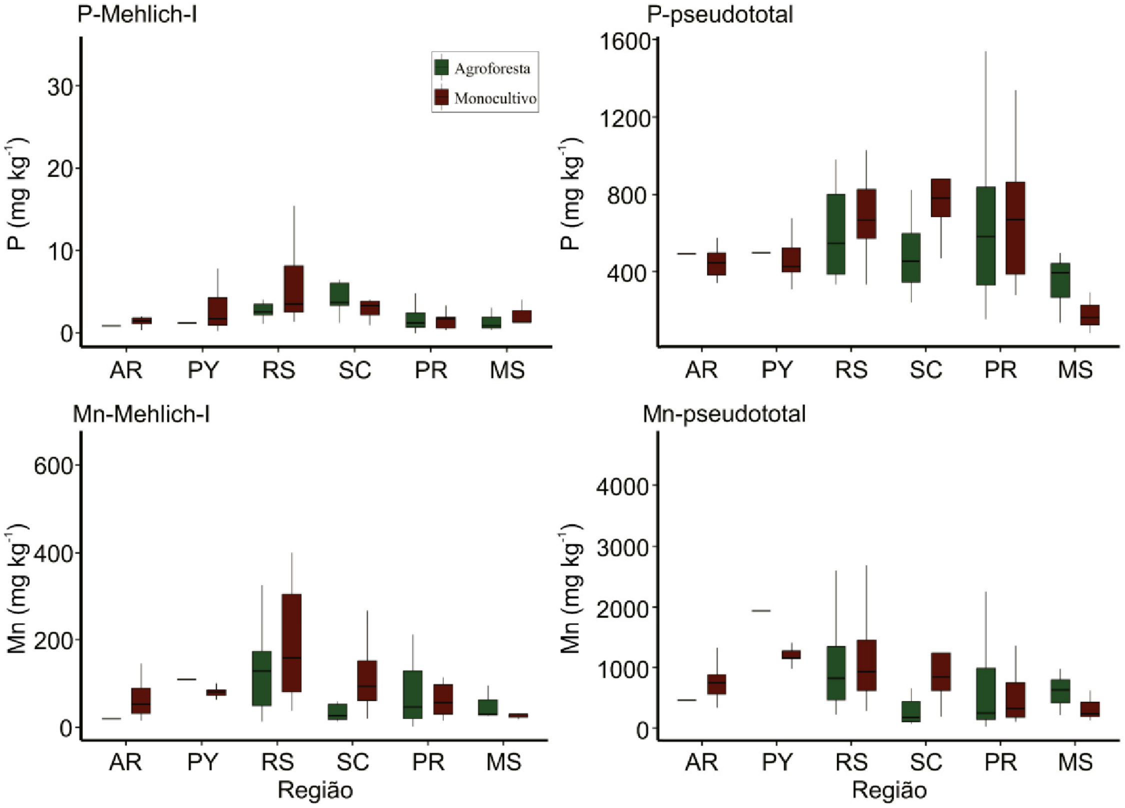
Region	**Fe**	**Al**	**Si**	**Mg**	**Mn**	**Ca**	**K**
	----- g kg^{-1} -----		-------------------------------- mg kg^{-1} --------------------------------				
			Basalt				
AR	121±19	102±22	2145±389	1052±301	774±365	491±344	1051±327
PY	125±22	84±19	1918±379	1110±166	1292±306	1690±1121	1559±328
RS	163±27	97±21	2595±290	1589±729	1465±616	1224±773	957±432
SC	123±41	109±30	2482±333	1674±1073	1066±1018	1018±1020	1053±416
PR	151±27	127±24	2231±256	1216±309	924±630	663±533	827±173
MS	161±23	109±9	1785±434	1042±347	744±200	409±308	584±190
			Rhyolite/rhyodacite				
RS	63±20	85±22	2452±130	1576±416	588±260	673±514	1211±299
PR	77±42	117±0,4	2505±36.6	1510±309	281±15	651±676	1021±264
			Sedimentary				
SC	28±9	71±19	2362±165	2373±709	125±51	486±451	2788±856
PR	48±22	97±39	2323±322	1439±748	500±1171	537±514	1652±905
MS	27±5	28±9	1258±28.2	294±121	200±55	371±206	364±210

Região	**P**	**S**	**Cu**	**Zn**	**B**	**Ba**	**Ni**
	-- mg kg^{-1} --						
			Basalt				
AR	446±70	245±32	139±58	66±19	75±14	79±50	25±7
PY	520±252	209±19	110±30	103±40	75±8	153±32	24±6
RS	781±148	278±57	217±92	107±32	93±13	93±88	36±18
SC	734±242	377±89	146±77	85±33	77±23	93±65	29±14
PR	904±281	449±134	209±107	89±31	92±17	71±39	35±12
MS	392±101	175±36	177±24	78±23	98±13	58±49	37±3
			Rhyolite/rhyodacite				
RS	502±170	304±47	47±37	56±13	38±10	107±22	6±3
PR	805±79	385±89	10±3	85±2	48±0.5	262±12	5±1
			Sedimentary				
SC	369±122	344±31	16±6	21±9	22±7	71±32	10±1
PR	371±172	305±59	30±23	28±19	30±13	52±44	12±6
MS	126±40	73±15	18±4	9±3	17±5	25±14	5±0,5

SOURCE: The author (2021).

LEGEND: RS= *Rio Grande do Sul* (N=35); SC= *Santa Catarina* (N=21), PR= *Paraná* (N=31); MS= *Mato Grosso do Sul* (N=6); AR= Argentina (N=12); PY= Paraguay (N=10).

FIGURE S1 – PHOSPHORUS AND MN CONCENTRATIONS IN SOILS (MEHLICH-I AND PSEUDO-TOTAL EXTRACTION) IN AGROFORESTRY AND MONOCULTURE SYSTEMS FROM THE MAIN YERBA MATE PRODUCING REGIONS IN SOUTH AMERICA (N=115).



SOURCE: The author (2021).

LEGEND: RS= *Rio Grande do Sul* (N=35); SC= *Santa Catarina* (N=21), PR= *Paraná* (N=31); MS= *Mato Grosso do Sul* (N=6); AR= Argentina (N=12); PY= Paraguay (N=10).

4 CHAPTER III: GLYPHOSATE EFFECTS ON NON-TARGET PLANTS: COLLATERAL IMPACTS ON ELEMENTAL COMPOSITION AND GROWTH OF YERBA MATE

4.1 RESUMO

Neste trabalho foi avaliado o efeito do uso de glifosato no crescimento e na composição elementar das folhas de erva-mate, especialmente dos elementos potencialmente tóxicos Cd e Pb. Foi conduzido um experimento em vasos com uso de glifosato (aplicação em braquiária; aplicação no solo; e controle) e com fornecimento de P (com e sem P), em dois cultivares de erva-mate clonal cultivados em dois solos distintos (originados de basalto e riodacito). O uso de glifosato nas braquiárias reduziu a matéria seca total e o diâmetro da base das plantas, com efeito potencializado pela carência de P no solo, e com diferença entre os clones e os solos. De maneira geral, o uso de glifosato nas braquiárias resultou no acréscimo de K, P, Cu em um dos clones quando cultivado em solo de basalto; nos teores de Ca, Mg, Mn, Fe e Zn (clone 1) e de B, Cd e Pb (clone 2) quando cultivado no solo de riodacito. Estas observações demonstram que ao usar o glifosato no controle de plantas indesejáveis pode ocorrer prejuízos ao desenvolvimento inicial da erva-mate, bem como alterar a sua composição elementar, com variações entre os cultivares e a origem do solo.

Palavras-chave: *Ilex paraguariensis.* Cádmio. Chumbo. Segurança alimentar. Material de origem do solo.

4.2 ABSTRACT

This work investigated collateral effects on non-target yerba mate (*Ilex paraguariensis* A. St.-Hil) plants when using glyphosate for weed control; growth and leaf elemental composition were examined. Special emphasis was placed on examining the heavy metals Cd and Pb due to regulations defining maximum limits in South American infusion products. The experiment was conducted in pots using glyphosate [applied to Congo grass (*Brachiaria ruziziensis*), soil surface and control], two P rates (with and without P), two clonal yerba mate cultivars, and two different soils (basalt- and rhyodacite-derived). When used to control Congo grass, glyphosate reduced total dry matter and base diameter of yerba mate plants; effects were enhanced by lack of P in soil and differed between clones and soils. Collateral effects of glyphosate use resulted in increased K, P, and Cu in one clone cultivated in basalt soil and increase levels of Ca, Mg, Mn, Fe and Zn in clone 1 and B, Cd and Pb in clone 2 when cultivated in rhyodacite soil. These observations demonstrate that glyphosate use to control undesirable plants can impact initial development and elemental composition of yerba mate, with variations between cultivars and soil type.

Keywords: *Ilex paraguariensis.* Cadmium. Lead. Food safety. Soil parent material.

4.3 INTRODUCTION

For centuries, yerba mate (*Ilex paraguariensis* A. St.-Hil) leaves have been exploited to produce infusion drinks in southern regions of South America. Historically, there was a predominance of extractivism of this species, which grows naturally in forest understories (Cardozo-Junior and Morand, 2016) under very acidic soil conditions (Reissmann et al., 1999; Toppel et al., 2018). However, increased consumption and consequent increase in demand for raw material has resulted in cultivations outside of native forests. Such commercial operations often use herbicides (especially glyphosate) and fertilizers to increase productivity. Due to positive yerba mate response, phosphate fertilization (Barbosa et al., 2018) has become popular among producers.

Worldwide, glyphosate is the most widely used herbicide (Beckie et al., 2020). This compound inhibits the activity of the enzyme 5-enolpyruvyl-shikimate-3-phosphate-synthase in the shikimate pathway and hinders synthesis of amino acids (tryptophan, phenylalanine and tyrosine), which leads to plant death (Zablotowicz and Reddy, 2004). In addition, C synthesis is disrupted and causes oxidative damage (Zobiole et al. 2011; Zobiole et al., 2012). When applied to plants, glyphosate penetrates cell interiors, is rapidly translocated due to high mobility in the phloem, and quickly reaches roots and apical meristems (Yamada and Castro, 2007). Glyphosate can be exuded by plant roots, which can promote changes in the rhizosphere and negatively impact soil microbiological communities (Lane et al., 2012; Kumar et al., 2017), especially colonizing mycorrhizae (Carvalho et al., 2014). Furthermore, Rodrigues et al. (1982) and Neumman et al. (2006) observed the absorption of glyphosate (via transfer through contact between root systems) by non-target plants from root exudates of target plants.

Glyphosate also reaches the soil via foliar wash and spray residue (Ellis and Griffin, 2002). Due to their high sorption capacity, glyphosate molecules can remain in soil as a residue by binding to humic substances, metallic atoms, and oxides (Yamada and Castro, 2007). However, there is evidence that simultaneous application with phosphate fertilizer can reduce soil sorption of glyphosate due to the preference of phosphate for binding sites (Pereira et al., 2019; Munira et al., 2016).

Studies evaluating nutrient accumulation in glyphosate-contaminated plants show a decrease in leaf Mn in sunflower (Tesfamariam et al., 2009) and soybean

(Duke et al., 2012a), and a reduction in Fe, Mn, Ca, and Mg in soybean seeds (Cakmak et al., 2009). Changes are attributed to these nutrients forming insoluble complexes with glyphosate that decrease nutrient absorption and translocation. França et al. (2010) reported a decrease in Mn, Fe, and P in coffee leaves (with different behavior among evaluated cultivars) when evaluating the effects of glyphosate on the nutrients N, P, K, Ca Mg, Fe, Zn, Mn, and Cu. Most other studies with coffee (Barbosa et al., 2020a), citrus (Gravena et al., 2012), *Camellia sinensis* (L.) (Rana et al., 2020), and *Eucalyptus grandis* (W. Hill ex Maiden.) (Pereira et al., 2019) have only evaluated glyphosate effects on plant growth or physiology.

The use of glyphosate and phosphate fertilizers raises concern regarding possible changes in elemental composition of yerba mate leaves, especially for the heavy metals Cd and Pb. Low concentrations of these potentially toxic elements are naturally present in yerba mate leaves (Valduga et al., 2019; Barbosa et al., 2020b; Frigo et al., 2020; Pardinho et al., 2020). In 2013, MERCOSUR (Common Market of the South) technical regulations defined maximum limits for Cd and Pb in teas and infusion drinks sold in South American countries. This legislation included yerba mate infusion products and set maximum limits of 0.40 and 0.60 mg kg^{-1} for Cd and Pb, respectively (Brasil, 2013). Research to date indicates that natural concentrations of Cd and Pb in mate leaves may be very close to or above maximum limits established by this legislation (Motta et al., 2020; Magri et al., 2021).

Since these elements can pose risks to human health (Boskabady et al., 2018; Hamid et al., 2019), investigating the presence of Cd and Pb in food crops is essential. In addition, no studies have evaluated the impacts and interaction of glyphosate and phosphate fertilizer use on levels of potentially toxic elements in yerba mate leaves. Our objective was to verify how glyphosate and phosphate fertilizer usage alters growth and leaf elemental composition (especially Cd and Pb) of yerba mate cultivars grown on two different soils (basalt and rhyodacite parent material).

4.4 MATERIALS AND METHODOS

4.4.1 EXPERIMENTAL DESIGN

An experiment was conducted as a 3 x 2 x 2 x 2 factorial consisting of 1) glyphosate application (three factors: applications to weeds, soil, and control), 2) phosphorus supply (two factors: with and without P), 3) two cultivars of clonal mate, and 4) two different soils (basalt and rhyodacite parent materials). There were five replications for each treatment.

Soils of igneous origin were collected from the state of *Rio Grande do Sul* in Brazil. Rhyodacite-derived and basalt-derived soils were collected from the municipalities of *Ilópolis* (28°54'50.89"S and 52°7'52.38"W) and *Barão de Cotegipe* (27°33'50.57"S and 52°24'4.01"W), respectively. Soils from these parent materials were selected since they represent regionally predominant types used in yerba mate production (Magri et al., 2021). The collection sites had no history of using glyphosate, fertilizers, or soil correctives.

Prior to soil collection, weeds and plant debris were removed from the soil surface. Soils were collected from the 0–20 cm depth and roughly screened in the field to remove roots and stones. Sieving (4 mm mesh) and homogenization was performed prior to placing 7 L of soil into 8 L plastic pots.

For phosphorus supply, solutions containing Dibasic Ammonium Phosphate [DAP; $(NH_4)_2HPO_4$] were added to each pot at rate of 64.93 mg kg^{-1} and homogenized before seedlings were planted. Since DAP has NH^{4+} in its formulation, urea ($CO(NH_2)_2$) was supplied to pots not receiving phosphate fertilization (0.98 g of urea per pot); this was equivalent to the N rate supplied by DAP. After this preparation, yerba mate clonal seedlings were planted in each pot; the two clonal cultivars (Clone 1: BR5–BLD Yary and Clone 2: BR5–BLD Aupaba) were propagated by the microcutting technique (Wendling et al., 2017). These seedlings were grown in 120 cm^3 tubes (125 mm high x 34 mm in diameter) and had attained heights of ~15 to 20 cm at transplanting. In December 2017, the experiment was initiated in an open experimental area located at the Agricultural Sciences Sector of the Federal University of Paraná, Curitiba-PR, Brazil. The experimental site had an altitude of ~910 m and a Cfb climate according to Köppen classification (Alvares et al., 2013).

Three-hundred fifty-four days after planting yerba mate seedlings, 40 Congo Grass (*Brachiaria ruziziensis*; Syn. *Urochloa ruziziensis*) seeds were sown to simulate a weed infestation. Commercial glyphosate (Monsanto Roundup Original® DI glyphosate, acid equivalent 370 g L^{-1}) was applied 48 days after planting Congo grass. For this procedure, a 15 mL L^{-1} glyphosate solution was prepared; from this solution, an equivalent of 2.5 L of glyphosate ha^{-1} was applied to each pot requiring an application using a calibrated CO_2 pressurized sprayer (set at a constant pressure of 250 kPa) equipped with a boom having two fan-type spray tips (50 cm apart). Although glyphosate is not labelled for yerba mate, the amount used was typical of that used in commercial yerba mate productions. For glyphosate application in weed and soil treatments, the base of yerba mate plants were wrapped with plastic to avoid contact with glyphosate. Glyphosate applications were carried out in a wind-free environment and were protected from water for 24 hrs to avoid washing the product from leaves. In the control treatment and in the treatment that received glyphosate to the soil, manual weeding was conducted periodically.

One-hundred sixty-three days after glyphosate application (~1.5 year from study initiation), stem diameters 2 cm above the ground were measured with digital calipers and plants were harvested. Six mature leaves of similar physiological age were also collected from the upper third of each plant canopy. These leaves were washed under running water and rinsed three times with distilled water. After air drying in a greenhouse (60° C recirculating air) until mass remained constant, total dry mass of each plant was determined.

4.4.2 SOIL AND PLANT ANALYSIS

Prior to fertilizer addition, samples were collected from each homogenized soil for chemical and granulometric characterization (TABLE 1). Samples were air-dried and disaggregated to pass through a 2 mm sieve. Granulometry of these samples was determined by the Bouyoucos hydrometer method using a mixture of sodium hydroxide (4 g L^{-1}) and sodium hexametaphosphate (10 g L^{-1}) as a dispersing solution (Gee and Or, 2002). Determinations of pH $CaCl_2$, exchangeable Al, H+Al, organic matter, and available levels of Mn, Fe, Zn, Cu, Ni and Ba were made using the methodologies of Marques and Motta (2003) (TABLE 1).

TABLE 1 – CHEMICAL AND PHYSICAL PROPRIETIES OF TWO SOILS PRIOR TO EXPERIMENT INITIATION.

Soil	**pH**	**Al^{3+}**	**H + Al**	**Ca^{2+}**	**Mg^{2+}**	**K^{+}**	**P**	**OM**
		--------------------- cmol$_c$ dm^{-3} ---------------------					mg kg^{-1}	%
Basalt	4.58	1.03	7.48	4.39	2.70	0.10	7.80	4.40
Rhyodacite	3.76	2.76	17.85	2.29	0.26	0.78	12.44	11.89
Soil	**Mn**	**Fe**	**Zn**	**Cu**	**Ni**	**Ba**	**Clay**	**Silt**
	------------------------------ mg kg^{-1} ------------------------------						g kg^{-1}	
Basalt	24	46	6.0	1.3	0.30	1.37	700	225
Rhyodacite	73	14	19.3	8.8	0.49	1.78	475	400

SOURCE: The author (2021).

LEGEND: Clay and silt (hydrometer method); pH (CaCl2 0.01 mol L^{-1}); Ca^{2+}, Mg^{2+}, Al^{3+} (KCl 1 mol L^{-1} extraction); H+Al^{3+} (calcium acetate 0.5 mol L^{-1} extraction); organic matter (OM; volumetric method by potassium dichromate); K^{+}, P, micronutrients and Ba (Mehlich-1 extraction).

Additionally, pseudo-total concentrations of Al, Ba, Ca, Cd, Cu, Fe, K, Mg, Mn, Ni, P, Pb, and Zn are shown in TABLE S1. For this determination, soil samples were again disaggregated and passed through a 0.21 mm sieve. This determination followed the USEPA 3051A methodology (USEPA, 1995) involving the digestion of 400 mg of soil in 9 mL of 65% nitric acid and 3 mL of 36–38% hydrochloric acid. Digestion was performed in a microwave oven (Mars 6 – CEM Corporation) for 4.5 min at a power setting between 1200–1800 W, a heating ramp of 5.5 min, and a maximum temperature of 175° C. After digestion, samples were brought to volume (50 mL) using ultra-pure water (Milli-Q system with resistance of 18.2 MΩ cm – Millipore Milli-Q Academic). Elements were quantified by inductively coupled plasma atomic emission spectrometry (ICP–OES, Varian 720–ES).

Leaf samples were crushed in a knife mill to pass through a 0.25 mm sieve. According to methodology described by Magri et al. (2021), leaf digestion was conducted in a microwave oven using 200 mg of sample, 4 mL of nitric acid, and 1 mL of hydrogen peroxide. Digestion of certified reference material (CRM – *C. sinensis* GBW 10052) and blank samples was also performed. Quantification of K, Ca, Mg, P, Mn, Al, Fe, Zn, Ba, B, Cu, and Ni was performed by ICP–OES. Quantification of Cd and Pb was conducted by atomic absorption with a graphite furnace – GFAAS (model AA 6800 – Shimadzu). Equipment parameters used followed those described by Magri et al. (2021). Recovery of quantified elements ranged from 74% to 104% (TABLE S2).

4.4.3 STATISTICAL ANALYSIS

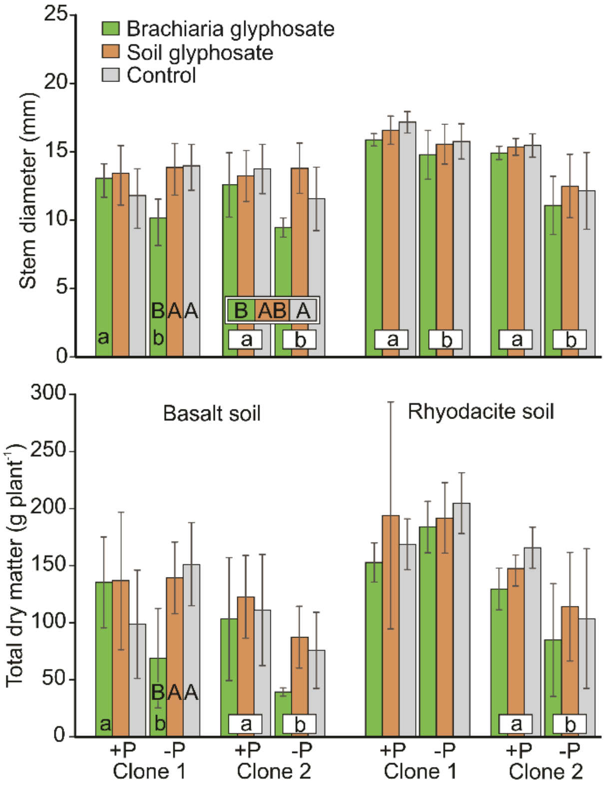
Data were tested for normality of residuals using the Shapiro-Wilk test. Variables not showing normality were transformed using Box-Cox transformation. Growth parameters and plant tissue elements were compared between treatments with glyphosate and with addition of phosphate fertilizer by mean comparison tests. This comparison was carried out individually for each clonal cultivar in each soil, adopting a two-way ANOVA followed by Tukey's test in cases of significant F ($p<0.05$). Additionally, from the correlation matrix of normalized and standardized data, principal component analysis and discriminant analysis were performed to verify differences between clonal cultivars and soil parent materials. Statistical analyses were performed using R software version 4.0.3 (R Core Team, 2020).

4.5 RESULTS

4.5.1 PLANT GROWTH ATTRIBUTES

Use of glyphosate to control Congo grass reduced basal stem diameter and total dry matter of yerba mate clone 1 grown in basalt soil without phosphate fertilization (FIGURE 1). A similar effect was also noted for stem diameter of clone 2 plants grown in the same soil, regardless of P addition. Although no statistical differences were identified for plants cultivated in rhyodacite derived-soil, yerba mate plants in treatments with glyphosate applied to Congo grass had the lowest stem diameter and total dry matter values. Furthermore, yerba mate plants from both clones and soils showed leaf yellowing after application of glyphosate to Congo grass.

FIGURA 1 – STEM BASAL DIAMETER AND DRY MATTER OF TWO YERBA MATE CLONES GROWN IN BASALT AND RHYODACITE SOILS SUBJECTED TO P FERTILIZATION AND GLYPHOSATE APPLICATION.



SOURCE: The author (2021).

LEGEND: Uppercase letters represent the effect of glyphosate treatments and lowercase letters represent the effect of phosphate fertilization: highlighted boxes – simple effect; letters on bars – interaction (two-way ANOVA, followed by Tukey test – p <0.05).

4.5.2 GLYPHOSATE AND PHOSPHORUS EFFECT ON ELEMENTARY COMPOSITION

When glyphosate was applied to Congo grass, clone 1 plants cultivated on basalt soil had higher K and P in leaves relative to the control (TABLE 2). When P was added, leaf Cu was smaller in the control compared to the two glyphosate treatments (TABLE 3). For clone 2 cultivated on basalt soil, treatments using glyphosate on Congo grass had higher leaf P compared to the control.

TABLE 2 – CONCENTRATION OF MACRONUTRIENTS IN LEAVES OF YERBA MATE CLONES IN RESPONSE TO GLYPHOSATE APPLICATION AND P FERTILIZATION.

	Basalt				Rhyodacite			
	Clone 1		**Clone 2**		**Clone 1**		**Clone 2**	
	+P	**-P**	**+P**	**-P**	**+P**	**-P**	**+P**	**-P**
K (g kg^{-1})								
Weed glyphosate	9.22A	11.35A	7.00	12.04	7.68	8,84	7,54	11,18
Soil glyphosate	7.53B	8.95B	6.28	10.05	7.50	8,32	7,69	10,50
Control	6.13B	7.57B	6.65	9.97	7.84	8,14	6,85	10,94
p-value glyphosate	<0.01*		0.30		0.70		0.87	
p-value phosphorus	0.01*		<0.01*		0.04*		<0.01*	
p-value interaction	0.87		0.64		0.62		0.76	
Ca (g kg^{-1})								
Weed glyphosate	6.20	5.23	6.89	5.37	6.48Aa	5,41ABb	6,67	4,76
Soil glyphosate	6.70	5.36	5.59	6.17	4.95Ba	4,73Ba	5,16	4,55
Control	6.12	5.81	5.86	5.57	5.65ABa	6,42Aa	5,50	4,31
p-value glyphosate	0.75		0.66		<0.01*		0.08	
p-value phosphorus	0.02*		0.28		0.53		<0.01*	
p-value interaction	0.50		0.09		0.03*		0.29	
Mg (g kg^{-1})								
Weed glyphosate	6.42	5.74	7.31Aa	5.61Ab	4.45A	4,26A	4,88	4,18
Soil glyphosate	6.56	5.88	6.37Aa	6.30Aa	3.76B	3,80B	4,27	4,09
Control	6.50	6.19	6.896Aa	6.30Ab	3.94AB	4,23AB	4,73	3,93
p-value glyphosate	0.51		0.88		0.02*		0.43	
p-value phosphorus	0.01*		<0.01*		0.76		0.12	
p-value interaction	0.63		0.02*		0.46		0.47	
P (g kg^{-1})								
Weed glyphosate	1.23A	1.40A	1.61A	1.76A	1.77	0,78	2,24	0,85
Soil glyphosate	0.92B	0.79B	1.23AB	1.53AB	1.53	0,72	2,45	0,62
Control	0.96B	0.71B	1.06B	0.85B	1.58	0,72	1,84	0,6
p-value glyphosate	0.01*		0.02*		0.58		0.21	
p-value phosphorus	<0.01*		0.68		<0.01*		<0.01*	
p-value interaction	0.59		0.55		0.82		0.33	

SOURCE: The author (2021).

LEGEND: Different values for uppercase letters (glyphosate effect) and lowercase (P effect) indicate significant difference by two-way ANOVA followed by Tukey test ($p<0.05$). The presence of an asterisk represents a significant F value.

TABLE 3 – CONCENTRATION OF MICRONUTRIENTS IN LEAVES OF YERBA MATE CLONES IN RESPONSE TO GLYPHOSATE USE AND P FERTILIZATION.

	Basalt				**Rhyodacite**			
	Clone 1		**Clone 2**		**Clone 1**		**Clone 2**	
	+P	**-P**	**+P**	**-P**	**+P**	**-P**	**+P**	**-P**
Mn (mg kg^{-1})								
Weed glyphosate	2047	2057	3082	2334	2413A	2404A	2066	2975
Soil glyphosate	2328	2600	2655	2777	1876B	1579B	1735	2133
Control	2377	2773	2622	3105	2302AB	2363AB	2059	2302
p-value glyphosate	0.07		0.87		0.03*		0.08	
p-value phosphorus	0.51		0.86		0.70		0.02*	
p-value interaction	0.85		0.18		0.76		0.38	
Fe (mg kg^{-1})								
Weed glyphosate	42	41	42	43	51A	47A	64	53
Soil glyphosate	58	31	44	49	45AB	39AB	58	49
Control	55	44	50	68	45B	34B	55	52
p-value glyphosate	0.43		0.80		0.04*		0.58	
p-value phosphorus	0.02*		0.62		0.03*		0.10	
p-value interaction	0.14		0.34		0.61		0.72	
Zn (mg kg^{-1})								
Weed glyphosate	50.1	48.4	74.2	49.8	30.5A	51.7A	41.4	37.4
Soil glyphosate	64.9	48.6	44.6	53.8	25.5B	32.3B	34.7	49.6
Control	62.2	50	43.1	50.7	23.9B	31.3B	22	36.9
p-value glyphosate	0.43		0.39		0.01*		0.38	
p-value phosphorus	0.06		0.79		<0.01*		0.27	
p-value interaction	0.50		0.29		0.22		0.52	
B (mg kg^{-1})								
Weed glyphosate	6.48	7.49	11.87	7.65	8.34±A	8.22A	9.7A	9.48A
Soil glyphosate	6.39	6.87	8.15	10.02	6.93B	6.79B	7.07AB	8.47AB
Control	5.82	7.38	10.11	8.12	7.8±A	8.52A	7.46B	7.92B
p-value glyphosate	0.78		0.81		0.01*		0.03*	
p-value phosphorus	0.05		0.14		0.69		0.38	
p-value interaction	0.68		0.05		0.57		0.56	
Cu (mg kg^{-1})								
Weed glyphosate	5.67Aa	3.44Ab	4.07	3.96	4.77	4.3	4.86	3.15
Soil glyphosate	4.87Aa	4.42Aa	4.13	4.36	5.15	4.81	4.82	3.84
Control	3.58Ba	3.73Aa	4.37	3.10	4.98	4.57	4.47	3.15
p-value glyphosate	0.02*		0.41		0.42		0.65	
p-value phosphorus	0.01*		0.22		0.15		0.01*	
p-value interaction	0.01*		0.13		0.98		0.81	
Ni (mg kg^{-1})								
Weed glyphosate	0.99	0.41	1.01	0.75	1.26	0.81	1.39	0.30
Soil glyphosate	1.05	0.80	0.96	0.77	1.73	0.96	1.59	0.69
Control	0.45	0.57	0.94	0.8	1.56	0.71	1.47	0.27
p-value glyphosate	0.09		0.8		0.62		0.72	
p-value phosphorus	0.22		0.13		0.01*		<0.01*	
p-value interaction	0.34		0.89		0.80		0.93	

SOURCE: The author (2021).

LEGEND: Different values for uppercase letters (glyphosate effect) and lowercase (P effect) indicate significant difference by two-way ANOVA followed by Tukey test ($p<0.05$). The presence of an asterisk represents a significant F value.

Compared to the control, clone 1 plants grown on rhyodacite-derived soil had higher levels of Ca (with added P), Mg, Mn, Fe, and Zn in treatments where glyphosate was applied to Congo grass (TABLE 2 and TABLE 3). Concentrations of Ba and B were lower in the treatment with glyphosate applied to soil compared to the control. Compared to the control, leaf levels of B, Cd, and Pb were higher for clone 2 in treatment where glyphosate was applied to Congo grass (TABLE 3 and TABLE 4). Differences in leaf Cd concentrations were greater when P was not added to soil.

TABLE 4 – CONCENTRATION OF AL, BA, CD, AND PB IN LEAVES OF YERBA MATE CLONES IN RESPONSE TO GLYPHOSATE USE AND P FERTILIZATION.

	Basalt				**Rhyodacite**			
	Clone 1		**Clone 2**		**Clone 1**		**Clone 2**	
	+P	**-P**	**+P**	**-P**	**+P**	**-P**	**+P**	**-P**
Al (mg kg^{-1})								
Weed glyphosate	171	182	217	192	294	323	305	249
Soil glyphosate	191	198	165	206	284	280	231	216
Control	185	205	169	220	299	314	247	241
p-value glyphosate	0.35		0.64		0.17		0.2	
p-value phosphorus	0.29		0.19		0.29		0.28	
p-value interaction	0.89		0.14		0.54		0.66	
Ba (mg kg^{-1})								
Weed glyphosate	35.4	38.3	49.8	43.8	38AB	32.2AB	39.4	38
Soil glyphosate	41.6	32.8	40.8	44.5	26.5B	30B	30.2	32
Control	42.5	37.6	51.9	35.7	32.9A	38.9A	33.7	24.7
p-value glyphosate	0.52		0.77		0.02*		0.13	
p-value phosphorus	0.16		0.22		0.58		0.47	
p-value interaction	0.16		0.26		0.10		0.52	
Cd (mg kg^{-1})								
Weed glyphosate	0.93	0.94	1.12	0.97	0.52	0.55	0.55±A	1.11A
Soil glyphosate	0.93	1.04	1.00	0.97	0.44	0.55	0.47B	0.62B
Control	0.90	0.98	1.03	1.02	0.51	0.5	0.51B	0.67B
p-value glyphosate	0.64		0.63		0.47		0.01*	
p-value phosphorus	0.16		0.20		0.16		<0.01*	
p-value interaction	0.67		0.54		0.18		0.06	
Pb (mg kg^{-1})								
Weed glyphosate	1.15	0.95	1.62	0.94	0.86	0.99	1.42A	1.50A
Soil glyphosate	1.28	1.08	1.24	1.10	0.78	1.05	1.26AB	1.23AB
Control	1.06	0.99	1.41	0.82	1.12	0.96	1.22B	1.11B
p-value glyphosate	0.47		0.45		0.56		0.01*	
p-value phosphorus	0.17		<0.01*		0.45		0.74	
p-value interaction	0.85		0.10		0.25		0.32	

SOURCE: The author (2021).

LEGEND: Different values for uppercase letters (glyphosate effect) and lowercase (P effect) indicate significant difference by two-way ANOVA followed by Tukey test ($p<0.05$). The presence of an asterisk represents a significant F value.

Phosphate fertilization resulted in a decrease in leaf K of both clones in both soils evaluated. Fertilization decreased leaf Zn in clone 1 cultivated on rhyodacite soil, while leaf Cd only decreased for clone 2 in this soil. When an effect was observed for the elements Ca, Mg, P, Mn, Fe, Cu, Ni and Pb, concentrations were higher in plants that received phosphate fertilization. Among these elements, we highlight the small Pb increase in clone 2 cultivated on basalt soil. In addition, Ni increased for both clones cultivated on rhyodacite soil, doubling for clone 1 and increasing ~three to five times for clone 2.

4.5.3 SOIL AND GENETIC EFFECTS ON ELEMENTAL COMPOSITION

Discriminant analysis had an efficiency of 87% when separating samples according to soils and clones used in the experiment. Discrimination took into account elemental levels of K, Ca, Mg, P, Mn, Zn, Al, Ni, Cd, and Pb. As seen in the discriminant analysis matrix (TABLE 5), soil was more sensitive than genetic variation in sample discrimination. Although samples were not efficiently discriminated by clone type, there was a clear difference in leaf elemental composition when considering the two soils used in this work.

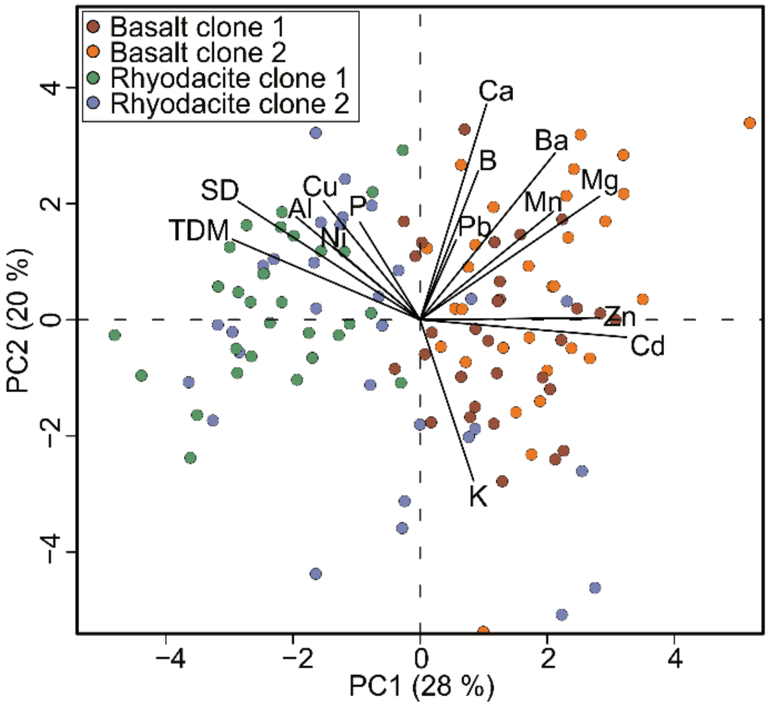
TABLE 5 – CONFUSION MATRIX OF DISCRIMINANT ANALYSIS – GLOBAL SOIL AND CLONE DISCRIMINATION (87% EFFICIENCY).

	Basalt clone 1	**Basalt clone 2**	**Rhyodacite clone 1**	**Rhyodacite clone 2**
Basalt clone 1	24	3	0	0
Basalt clone 2	6	27	0	0
Rhyodacite clone 1	0	0	28	5
Rhyodacite clone 2	0	0	2	25

SOURCE: The author (2021).

These differences can also be observed in raw values (TABLE 2, TABLE 3 and TABLE 4) and in the principal components analysis (FIGURE 2 and TABLE S3). Leaf concentrations of Mg, Zn, and Cd were higher in plants grown on basalt soil, while Al and Ni were higher when cultivated on rhyodacite soil. Calcium and Mn concentrations were also higher for basalt soils, but this effect was restricted to clone 2. Regarding comparison between clones, the main difference higher P and K in clone 2 (TABLE 2).

FIGURE 2 – PRINCIPAL COMPONENT ANALYSIS OF FOLIAR ELEMENTAL COMPOSITION OF TWO YERBA MATE CLONES GROWN ON BASALT AND RHYODACITE SOILS SUBJECTED TO P FERTILIZATION AND GLYPHOSATE APPLICATION.



SOURCE: The author (2021).

LEGEND: SD= Stem diameter of plant; TDM= Total dry matter of plant.

4.6 DISCUSSION

According to Eker et al. (2006), observed yellowing of yerba mate plants after applying glyphosate to Congo grass may be associated with Fe and Mn inactivation due to accumulation of glyphosate in leaf tissue. This process may occur due to the high capacity of glyphosate to bond with divalent cations, resulting in immobilization in plant tissue (Bellaloui et al., 2009; Cakmak et al., 2009; Zobiole et al., 2011). In glyphosate-resistant soybean plants, yellowing after use is also common (Zablotowicz and Reddy, 2007). Rather than a soybean nutritional imbalance, toxicity from aminomethylphosphonic acid (main metabolite generated in the degradation of glyphosate; Duke et al., 2012b) affects the synthesis of chlorophyll (Reddy et al., 2004; Serra et al., 2013).

Uptake of glyphosate by both leaves and roots, as well as mobility in the phloem and xylem, is directly affected by the availability of P (Pereira et al., 2019). When plants are grown under low P availability, increased expression of high affinity P transporters is induced (Gu et al., 2016). Studies indicate that these transporters are responsible for transporting glyphosate within plants (Fitzgibbon and Braymer 1988; Pereira et al., 2019). Under low P availability in our study, glyphosate was likely absorbed in greater amounts by Congo grass, translocated and exuded in greater quantity by their roots, and absorbed in greater quantity by yerba mate roots. Since the clay content in soil formed from basalt is much higher than in rhyodacite-derived soil, more P was adsorbed and not readily available to plants. For this reason, growth effects associated with P use were more pronounced for the basalt soil.

Plant uptake of glyphosate from soil is practically nil, due to the high adsorption force on soil colloids (Tesfamariam et al., 2009; Neumann et al., 2006; Bott et al., 2011). However, there is no doubt concerning resorption after exudation by plants treated with glyphosate (Rodrigues et al., 1982; Neumman et al., 2006). Thus, contact between Congo grass roots and yerba mate roots possibly allowed transport and absorption of glyphosate by yerba mate. Thus, effects on growth and elemental composition were more pronounced when using glyphosate on Congo grass than when applied directly to soil.

The occurrence of chlorosis without nutritional deficiency was indicative of glyphosate absorption by yerba mate plants. Santos et al. (2008) identified the presence of glyphosate in all eucalyptus plants intercropped with Congo grass that received glyphosate, but not in sufficient quantity to cause injury. Tong et al. (2017) evaluated the uptake and translocation of glyphosate in *C. sinensis* plants grown in hydroponic solution, and found that glyphosate was absorbed by roots and transferred to leaves. This process occurred from application until the fifth day when glyphosate concentration in leaves began to decrease. This implies that tea consumers can be exposed to glyphosate when applied a few days before harvest. Specific studies are required to determine if glyphosate is present in yerba mate leaves. If true, wait periods between glyphosate application and leaf harvest may need to be determined.

In the present study, glyphosate application to control Congo grass did not decrease leaf concentration of any evaluated element (TABLE 2, TABLE 3 and TABLE 4). However, changes in plant elemental composition caused by glyphosate use is very contradictory since some studies indicated decreased elemental concentrations (França et al., 2010; Cakmak et al., 2009), increased concentrations (Zobiole et al., 2010; 2011; 2012), or no concentration change (Bailey et al., 2002;

Rosolem et al., 2010). Additionally, similar work with soybeans indicated both increases and decreases in elemental concentrations (especially metals) that primarily varied by cultivar (Cavalieri et al., 2012). Collectively, findings indicate large variations between species or even between cultivars. The use of yerba mate cultivars is still incipient, since cultivation are predominantly produced from seeds and plants extracted from native habitats. Thus, large variation in collateral effects of glyphosate use on elemental composition of field-grown yerba mate can be expected.

Considering the potentially toxic elements Cd and Pb, there is a concern about their presence in yerba mate leaves since it is classified as a food and fall under specific legislation. However, even control treatments had values (TABLE 4) above those established by legislation (Brasil, 2013). Additionally, even with observed alterations due to glyphosate use, values were lower than natural accumulation potentials (Magri et al., 2021). These authors indicated that foliar Cd was dependent on Zn, as was also shown in FIGURE 2. In addition, intake of Cd and Pb by yerba mate consumers was very low, indicating that legislation governing elemental levels in yerba mate products is inconsistent. Another important factor to consider is that soil type and genetic factors have much more influence on Cd and Pb levels than does the use of glyphosate.

4.7 CONCLUSION

Observations from our study demonstrate that use of glyphosate to control unwanted plants can harm initial development of yerba mate on P-deficient soils, with distinct effects noted between clonal cultivars and type of soil in which plant were grown. Elemental composition of yerba mate leaves can be altered, as reflected by increases in some elements that differed between cultivars and soil type. Regarding the potentially toxic elements Cd and Pb, there were small increases in some cases, but these changes were not expressive to point of being a matter of concern to producers and consumers.

4.8 REFERENCES

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M. & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22(6), 711–728.

Bailey, W. A., Poston, D. H., Wilson, H. P. & Hines, T. E. (2002). Glyphosate interactions with manganese. *Weed Technology*. 16(4), 792-799.

Barbosa, E. A., Silva, I. M. D., França, A. C., Silva, E. D. B. & Matos, C. D. C. D. (2020a). Evaluation of leaf and root absorptions of glyphosate in the growth of coffee plants. *Arquivos do Instituto Biológico*, 87, 1-8.

Barbosa, J. Z., Motta, A. C., Consalter, R., Poggere, G. C., Santin, D., & Wendling, I. (2018). Plant growth, nutrients and potentially toxic elements in leaves of yerba mate clones in response to phosphorus in acid soils. *Anais da Academia Brasileira de Ciências*, 90(1), 557-571

Barbosa, J. Z., Motta, A. C. V., dos Reis, A. R., Corrêa, R. S., & Prior, S. A. (2020b). Spatial distribution of structural elements in leaves of *Ilex paraguariensis*: physiological and ecological implications. *Trees*, 34(1), 101-110.

Beckie, H. J., Flower, K. C. & Ashworth, M. B. (2020). Farming without Glyphosate? *Plants*, 9(1), 96.

Bellaloui, N., Reddy, K. N., Zablotowicz, R. M., Abbas, H. K. & Abel, C. A. (2009). Effects of glyphosate application on seed iron and root ferric (III) reductase in soybean cultivars. *Journal of Agricultural and Food Chemistry*, 57, 9569-9574.

Boskabady, M., Marefati, N., Farkhondeh, T., Shakeri, F., Farshbaf, A. & Boskabady, M. H. (2018). The effect of environmental lead exposure on human health and the contribution of inflammatory mechanisms, a review. *Environment international*, 120, 404-420.

Bott, S., Tesfamariam, T., Kania, A., Eman, B., Aslan, N., Romheld, V. & Neumann, G. (2011). Phytotoxicity of glyphosate soil residues re-mobilised by phosphate fertilization. *Plant Soil*, 342:249–263

Brasil. (2013). Dispõe sobre o Regulamento Técnico MERCOSUL sobre Limites Máximos de Contaminantes Inorgânicos em Alimento (Resolução-RDC nº 42, de 29 de agosto de 2013). Diário Oficial [da] República Federativa do Brasil.

Cakmak, I., Yazici, A., Tutus, Y. & Ozturk, L. (2009). Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. *European Journal of Agronomy*, 31(3), 114-119.

Cardozo-Junior, E. L. & Morand, C. (2016). Interest of mate (*Ilex paraguariensis* A. St.-Hil.) as a new natural functional food to preserve human cardiovascular health – A review. *Journal of Functional Foods*, 21, 440-454.

Carvalho, F. P., Souza, B. P., França, A. C., Ferreira, E. A., Franco, M. H. R., Kasuya, M. C. M. & Ferreira, F. A. (2014). Glyphosate drift affects arbuscular mycorrhizal association in coffee. *Planta Daninha*, 32(4), 783-789.

Castanheira, D. T., Alecrim, A. D. O., Voltolini, G. B., Rezende, T. T., Menicucci-Netto, P. & Guimarães, R. J. (2019). Growth, anatomy and physiology of coffee plants intoxicated by the herbicide glyphosate, *Coffee Science*, 14(1), 76-82.

Cavalieri, S. D., Velini, E. D., Silva, F. M. L., São José, A. R., Andrade & G. J. M. (2012). Acúmulo de nutrientes e matéria seca na parte aérea de dois cultivares de soja RR sob efeito de formulações de glyphosate. *Planta daninha*, 30(2), 349-358.

Duke, S. O., Lyndon, J., Koskinen, W. C., Moorman, T. B., Chaney, R. L. & Hammerschmidt, R. (2012a). Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. *Journal of agricultural and food chemistry*, 60, 10375-10397.

Duke, S. O., Reddy, K. N, Bu, K. & Cizdziel, J. V., (2012b). Effects of glyphosate on the mineral content of glyphosate resistant soybeans (*Glycine max*). *Journal of Agricultural and Food Chemistry*, 60, 6764–6771.

Duke, S. O. (2018). The history and current status of glyphosate. Pest management science, 74(5), 1027-1034.

Duke, S. O. & Reddy, K. N. (2018). Is mineral nutrition of glyphosate-resistant crops altered by glyphosate treatment? *Outlooks on Pest Management*, 29(5), 206-208.

Eker, S., Ozturk, L., Yazici, A., Erenoglu, B., Romheld, V. & Cakmak, I. (2006). Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. *Journal of Agricultural and Food Chemistry*. 54, 10019–10025.

Ellis, J. M. & Griffin, J. L. (2002). Soybean (*Glycine max*) and cotton (Gossypium hirsutum) response to simulated drift of glyphosate and glufosinate. *Weed Technology*, 16, 580-586.

Fitzgibbon, J. & Braymer, H. D. (1988). Phosphate starvation induces uptake of glyphosate by *Pseudomonas sp.* strain PG2982. *Applied and environmental microbiology*, 54:1886–1888

França, A. C., Freitas, M. A. M., D'Antonino, L., Fialho, C. M. T., Silva, A. A., Reis, M. R. & Ronchi, C. P. (2010). Teores de nutrientes em cultivares de café arábica submetidos à deriva de glyphosate. *Planta daninha*, 28(4), 877-885.

Frigo, C., Magri, E., Barbosa, J. Z., Sarteretto, L. M., Araujo, E. M., Prior, S. A., & Motta, A. C. V. (2020). Influence of roadways on heavy metal content in soils and yerba mate tissue in southern Brazil. *Management of Environmental Quality: An International Journal*, 31 No. 6, pp. 1477-1495. https://doi.org/10.1108/MEQ-10-2019-0219.

Gee, G.W. & Or, D. (2002). 2.4 Particle-size analysis*. Methods of soil analysis: Part 4 physical methods*, 5, 255-293.

Gu, M., Chen, A., Sun, S., Xu, G. (2016). Complex regulation of plant phosphate transporters and the gap between molecular mechanisms and practical application: what is missing? *Molecular Plant*, 9:396–416

Gravena, R., Filho, R. V., Alves, P. L. C., Mazzafera, P. & Gravena, A. R. (2012). Glyphosate has low toxicity to citrus plants growing in the field. *Canadian Journal of Plant Science*, 92(1), 119-127.

Hamid, Y., Tang, L., Sohail, M. I., Cao, X., Hussain, B., Aziz, M. Z., Usman, M., He, Z. & Yang, X. (2019). An explanation of soil amendments to reduce cadmium phytoavailability and transfer to food chain. *Science of The Total Environment*, 660, 80-96.. https:// doi.org/10.1016/j.scitotenv.2018.12.419.

Kumar, A., Ghosh, A., Mondal, D., Ghosh, R. & Bandopadhyay, P. (2017). Bio-efficacy of ammonium salt of Glyphosate 71% SG for weed dynamics in tea (*Camellia sinensis* L.) and its effect on soil microflora. *International Journal of Current Microbiology and Applied Sciences*, 6, 2160-2170.

Lane, M., Lorenz, N., Saxena, J., Ramsier, C. & Dick, R. P. (2012). The effect of glyphosate on soil microbial activity, microbial community structure, and soil potassium. *Pedobiologia*, 55(6), 335-342.

Magri, E., Valduga, A. T., Gonçalves, I. L., Barbosa, J. Z., de Oliveira Rabel, D., Menezes, I. M. N. R., Nascimento, P. A., Oliveira, A., Corrêa, R. S. & Motta, A. C. V. (2021). Cadmium and lead concentrations in yerba mate leaves from agroforestry and plantation systems: An international survey in South America. *Journal of Food Composition and Analysis*, 96, 103702.

Marques, R. & Motta, A. C. V. (2003). Análise química do solo para fins de fertilidade. In: Lima, M.R.de. (Ed.), *Manual de diagnóstico da fertilidade e manejo dos solos agrícolas, 2nd ed.* Departamento de Solos e Engenharia Agrícola, Curitiba, pp. 81–102.

Motta, A. C. V., Barbosa, J. Z., Magri, E., Pedreira, G. Q., Santin, D., Prior, S. A., Consalter, R., Young, S. D., Broadley, M. R. & Benedetti, E. L. (2020). Elemental composition of yerba mate (*Ilex paraguariensis* A. St.-Hil.) under low input systems of southern Brazil. *Science of the Total Environment*, 736, 139637.

Munira, S., Farenhorst, A., Flaten, D. & Grant, C. (2016). Phosphate fertilizer impacts on glyphosate sorption by soil. *Chemosphere*, 53: 471-477.

Neumann, G., Kohls, S., Landsberg, E., Stock-Oliveira Souza, K., Yamada, T. & Romheld, V. (2006). Relevance of glyphosate transfer to non-target plants via the rhizosphere. *Journal of Plant Diseases and Protection*. 20: 963-969.

Pardinho, R. B., Vecchia, P. D., Alves, C. M., Pimentel, N., Gazzana, D., Bolzan, R. C. & Flores, E. M. (2020). *Ilex paraguariensis* exposition to As and Cd in a closed soilless system. *Chemosphere*, 258, 127284.

Pereira, F. C. M., Tayengwa, R., Alves, P. L. D. C. A. & Peer, W. A. (2019). Phosphate Status Affects Phosphate Transporter Expression and Glyphosate Uptake and Transport in Grand Eucalyptus (*Eucalyptus grandis*). Weed Science, 67(1), 29-40.

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rana, S. S., Kumar, R., Kumar, S., Sharma, N. & Badiyala, D. (2020). Evaluation of glyphosate against weeds and phytotoxicity, productivity and soil quality parameters in tea (*Camellia sinensis* O. kuntze). *Journal of Research in Weed Science*, 3(1), 90-100.

Reddy, K. N., Rimando, A. M., Duke, S. O. (2004). Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean. *Journal of Agricultural and Food Chemistry*, 52, 5139–5143.

Reissmann, C. B., Radomski, M. I. & Quadros, R. M. B. (1999). Chemical composition of *Ilex paraguariensis* St. Hil. under different management conditions in seven localities of Paraná State. *Brazilian Archives of Biology and Technology*, 42(2), 187–194.

Rodrigues, J. J., Worsham, A. D. & Corbin, F. T. (1982). Exudation of glyphosate from wheat (*Triticum aestivum*) plants and its effects on interplanted corn (*Zea mays*) and soybeans (*Glycine max*). Weed Science, 316-320.

Rosolem, C. A., Andrade, G. J. M. D., Lisboa, I. P. & Zoca, S. M. (2010). Manganese uptake and redistribution in soybean as affected by glyphosate. *Revista Brasileira de Ciência do Solo*, 34(6), 1915-1922.

Santos, L. D. T., Santos, J. B., Ferreira, F. A., Oliveira, J. A., Bentivenha, S. & Machado, A.F.L. (2008). Exsudação radicular de glyphosate por Brachiaria decumbens e seus efeitos em plantas de eucalipto. *Planta Daninha*, 26(2), 369-374.

Serra, A. A., Nuttens, A., Larvor, V., Renault, D., Couée, I., Sulmon, C. & Gouesbet, G. (2013). Low environmentally relevant levels of bioactive xenobiotics and associated degradation products cause cryptic perturbations of metabolism and molecular stress responses in Arabidopsis thaliana. *Journal of Experimental Botany*, 64, 2753–2766.

Tesfamariam, T., Bott, S., Cakmak, I., Römheld, V. & Neumann, G. (2009). Glyphosate in the rhizosphere - Role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants. *European journal of agronomy*, 31, 126-132.

Toppel, F. V., Junior, A. M., Motta, A. C. V., Frigo, C., Magri, E. & Barbosa, J. Z. (2018). Soil chemical attributes and their influence on elemental composition of yerba mate leaves. *Floresta*, 48(3), 425-434.

Tong, M., Gao, W., Jiao, W., Zhou, J., Li, Y., He, L. & Hou, R. (2017). Uptake, translocation, metabolism, and distribution of glyphosate in nontarget tea plant (*Camellia sinensis* L.). *Journal of agricultural and food chemistry*, 65(35), 7638-7646. doi:10.1021/acs.jafc.7b02474.

Usepa, E. (1995). Method 3051: Microwave assisted acid digestion of sediments, sludges, soils, and oils. Test methods for evaluating solid waste, pp 1-30.

Valduga, A. T., Gonçalves, I. L., Magri, E. & Finzer, J. R. D. (2019). Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. *Food Research International*. Int. 120, 478–503.

Yamada, T. & Castro, P. R. D. C. (2007). Efeitos do glifosato nas plantas: implicações fisiológicas e agronômicas. *Informações Agronômicas*, 119, 1-32.

Zablotowicz, R. M. & Reddy, K. N. (2007). Nitrogenase activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean. *Crop Protection*, 26, 370-376.

Zablotowicz, R. M. & Reddy, K. N. (2004). Impact of glyphosate on the Bradyrhizobium japonicum symbiosis with glyphosate-resistant transgenic soybean. *Journal of Environmental Quality*, 33, 825-831.

Zobiole, L. H. S., Kremer, R. J., Oliveira, R. S. J. & Constantin, J. (2012). Glyphosate effects on photosynthesis, nutrient accumulation, and nodulation in glyphosate-resistant soybean. *Journal of Plant Nutrition and Soil Science*, 175, 319–330.

Zobiole, L. H. S., Kremer, R. J., Oliveira, R. S. J., Constantin, J. & Oliveira, R. S. (2011). Glyphosate affects chlorophyll, nodulation and nutrient accumulation of “second generation” glyphosate-resistant soybean (*Glycine max* L.). *Pesticide Biochemistry and Physiology*, 99, 53–60.

Zobiole, L. H. S., Oliveira, R. S., Kremer, R. J., Constantin, J., Yamada, T., Castro, C., Oliveira, F. A. & Oliveira, A. (2010). Effect of glyphosate on symbiotic N2 fixation and nickel concentration in glyphosate-resistant soybeans. *Applied Soil Ecology*, 44, 176–180.

4.9 SUPPLEMENTARY MATERIAL

TABLE S1 – PSEUDO-TOTAL ELEMENTAL CONCENTRATIONS IN TWO SOILS USED IN THE EXPERIMENT (mg kg^{-1}).

Soil	Fe	Al	Ca	Mn	K	Mg	P	Cu	Zn	Ba	Ni	Pb	Cd
Basalt	181,664	119,214	11,199	2,579	2,443	2,272	1,413	218	161	64	33.3	18.91	1.39
Rhyodacite	70,929	70,883	8,482	886	2,829	1,985	528	20	86	60	nd.	18.09	0.39

SOURCE: The author (2021).

LEGEND: nd= Non-detected.

TABLE S2 – MEASURED AND CERTIFIED VALUES (mg kg^{-1}) OF CERTIFIED REFERENCE MATERIAL – CRM (*Camellia sinensis* GBW 10052).

Element	Value measured	Value certified	Recovery (%)
K	12,814	15,500	83
Ca	10,583	12,100	87
Mg	1,979	2,200	90
P	2,460	2,800	88
Mn	1,201	1,170	103
Al	1,044	-	-
Fe	245	322	76
Zn	26	35	74
Ba	34	41	83
B	3.29	-	-
Cu	21	24	88
Ni	4.87	5.4	90
Cd	0.079	0.076	104
Pb	1.59	1.6	99

SOURCE: The author (2021).

TABLE S3 – ENGIVECTOR AND ENGIVALUE OF PRINCIPAL COMPONENT ANALYSIS OF FOLIAR ELEMENTAL COMPOSITION OF TWO YERBA MATE CLONES GROWN ON BASALT AND RHYODACITE SOILS SUBJECTED TO P FERTILIZATION AND GLYPHOSATE USE.

Variable	**CP1**		**CP2**		**CP3**		**CP4**	
	Eigenvector	σ^2 (%)	Eigenvector	σ^2 (%)	Eigenvector	σ^2 (%)	Eigenvector	σ^2 (%)
Al	-0.25	6	0.21	5	0.42	18	0.31	9
B	0.11	1	0.32	10	0.35	12	0.36	13
Ba	0.26	7	0.36	13	-0.01	0	0.06	0
Ca	0.12	2	0.47	22	-0.06	0	-0.07	0
Cd	0.41	16	-0.03	0	-0.08	1	0.01	0
Cu	-0.19	4	0.25	6	-0.32	10	0.35	12
K	0.11	1	-0.34	12	0.01	0	0.53	28
Mg	0.35	12	0.27	7	-0.21	5	-0.21	4
Mn	0.26	7	0.24	6	0.41	17	0.03	0
Ni	-0.17	3	0.15	2	-0.37	14	0.45	21
P	-0.12	2	0.21	4	-0.44	19	0.03	0
Pb	0.01	0	0.21	4	-0.10	1	-0.18	3
Zn	0.35	12	0.02	0	-0.11	1	0.15	2
SD	-0.36	13	0.24	6	0.10	1	-0.13	2
TDM	-0.37	14	0.16	3	0.10	1	-0.20	4
Eigenvalue	2.04		1.73		1.3		1.18	
% explained	28		20		11		9	
% accumulated	28		48		59		68	

SOURCE: The author (2021).

LEGEND: SD= Stem diameter of plant; TDM= Total dry matter of plant.

5 CAPÍTULO IV: MANGANESE HYPERACCUMULATION CAPACITY OF *Ilex paraguariensis* A. ST. HIL. AND OCCURRENCE OF INTERVEINAL CHLOROSIS INDUCED BY TRANSIENT TOXICITY*

5.1 RESUMO

A toxicidade por manganês (Mn) é comum em plantas cultivadas em solos muito ácidos. Contudo, algumas espécies de plantas que crescem nestas condições podem absorver grandes quantidades de Mn e são chamadas de espécies hiper acumuladoras. Neste estudo, nós avaliamos a capacidade da *Ilex paraguariensis* em acumular Mn e o efeito da concentração excessiva no crescimento da planta e nutrição. Para isto, um experimento em vasos foi conduzido usando solos de diferentes materiais de origem (basalto e arenito), com e sem calagem; e foram aplicadas seis doses de Mn (0, 30, 90, 270, 540 e 1080 mg kg^{-1}). Plantas clonais cultivadas por 203 dias foram colhidas para avaliar produção, e amostras do tecido foliar foram avaliadas quanto ao Mn e outros elementos. Sem calagem e com altas doses de Mn, a concentração foliar de Mn atingiu 13.452 e 12.127 mg kg^{-1} nos solos de arenito e basalto, respectivamente; concentração acima de 10.000 mg kg^{-1} são características de plantas hiper acumuladoras. A calagem reduziu estes valores para 7.203 e 8.030 mg kg^{-1}. O crescimento das plantas acompanhou o aumento da concentração de Mn foliar, com redução no crescimento observada na maior dose de Mn em solos sem calagem. A distribuição elementar mostrou a presença de Mn no mesófilo, principalmente nos feixes vasculares, sem ocorrência de precipitados de Mn. Foi observado clorose internerval nas folhas jovens, associada a alta concentração de Mn e baixa concentração de Fe, especialmente no solo de arenito sem calagem. Contudo, este sintoma não esteve associado a redução no crescimento das plantas.

Palavras-chave: Erva-mate. Desequilíbrio nutricional. Composição elementar. Microanálise de raio-X.

5.2 ABSTRACT

Manganese (Mn) toxicity is common in plants grown on very acid soils. However, some plants species that grow in this condition can take up high amounts of Mn and are referred to as hyperaccumulating species. In this study, we evaluated the capacity of *Ilex paraguariensis* to accumulate Mn and the effect of excessive concentrations on plant growth and nutrition. For this, a container experiment was conducted using soils from different parent materials (basalt and sandstone), with and without liming; and at six doses of applied Mn (0, 30, 90, 270, 540 and 1,080 mg kg^{-1}). Clonal plants grown for 203 days were harvested to evaluate yield, and leaf tissue samples were evaluated for Mn and other elements. Without liming and with high Mn doses, leaf Mn concentrations reached 13,452 and 12,127 mg kg^{-1} in sandstone and basalt soils, respectively; concentrations in excess of 10,000 mg kg^{-1}

are characteristic of hyperaccumulating plants. Liming reduced these values to 7,203 and 8,030 mg kg^{-1}. More plant growth accompanied increased Mn leaf concentrations, with a growth reduction noted at the highest dose in unlimed soils. Elemental distribution showed Mn presence in the mesophyll, primarily in vascular bundles, without high Mn precipitates. Interveinal chlorosis of young leaves associated with high Mn concentration and lower Fe concentrations was observed, especially in sandstone soil without liming. However, the occurrence of this symptom was not associated with decreased plant grow.

Keywords: Yerba mate. Nutritional imbalance. Elemental composition. X-ray microanalysis.

5.3 INTRODUCTION

Manganese (Mn) is a plant micronutrient taken up by roots in active or passive forms in the Mn^{2+} oxidation state (Broadley et al., 2012). Functional roles are related to the oxygen evolution complex that aids water photolysis in photosystem-II, chlorophyll synthesis, and activation of numerous enzymes (Schmidt et al., 2016). In vegetative tissues, Mn can accumulate in exchangeable forms, adsorb to negative charges of cell walls, and can exist in available (in cytoplasm) and unavailable (in vacuoles) forms (Junior et al., 2008).

In soil, this micronutrient is the most abundant following iron (Fe) and is mainly found in Mn^{2+} and Mn^{4+} oxidation states, with total amounts varying from 200 to 3,000 mg kg^{-1} (Oliva et al., 2014; Kabata-Pendias, 2010). Manganese plant availability can be altered by pH changes, and is more available in acidic soils, which produces favorable conditions for phytotoxicity in plants (Millaleo et al., 2010; Broadley et al., 2012). In addition, soils derived from igneous sources such as basalt have higher Mn values compared to sedimentary-based soils such as sandstone (Althaus et al., 2018). This difference can affect the amount of Mn availability and uptake by plants (Motta et al., 2020).

Some plants species have the capacity to accumulate high amounts of Mn in tissue without expressing toxicity symptoms or affecting growth. Some species can accumulate leaf concentrations in excess of 1,000 mg kg^{-1}, such as 5,996 mg kg^{-1} in *Phytolacca americana* L. (Phytolaccaceae) (Zhao et al., 2012) and 5,973 to 6,924 mg kg^{-1} in *Alternanthera philoxeroides* (Mart.) Griseb. – Amaranthaceae (Xue et al., 2004). On the other hand, some species are known as hyperaccumulators because they can accumulate Mn in excess of 10,000 mg kg^{-1} (Baker and Brooks, 1989). In a global review of metal hyperaccumulator plants, Reeves et al. (2018) discussed 24 species that presented Mn concentration up to 10,000 mg kg^{-1}. In South America, the tree species *Ilex paraguariensis* A. St. Hil. (Aquifoliaceae) is reported to have a high capacity for accumulating Mn in leaves, with concentrations usually close or up to 1,000 mg kg^{-1} (Reissmann and Carneiro, 2004; Oliva et al., 2014; Barbosa et al., 2018, Barbosa et al., 2020). Motta et al. (2020) reported Mn concentration of 9,401 ± 1,591 mg kg^{-1} in leaves of *I. paraguariensis* under typical forest conditions, and suggested future research to better explore the Mn accumulation potential of this species.

Another important aspect is that *I. paraguariensis* leaves are used to prepare hot or cold water infusions for human consumption (i.g., “*chimarrão*”, “*terrerê*”, and various teas) (Valduga et al., 2019). Barbosa et al. (2015) reported that up to 45% of Mn in *I. paraguariensis* leaves can be extracted via hot water infusion. Therefore, attention should be paid to the amount of Mn present in raw material used for yerba mate infusion preparations, since absorption of Mn above recommended maximum amounts can result in neurotoxic effects, higher incidence of acute bronchitis, bronchial asthma, and pneumonia (Leite et al., 2014).

Studies have indicated that Mn is the main micronutrient affected by liming *I. paraguariensis*, where a 57% decrease in Mn leaf concentration has been reported (Reissmann et al., 1999). Santin et al. (2013) reported a similar decrease in leaves (~50%), a high decrease in shoots, and no effect in roots. These evaluations reflect normal field conditions, but behaviors under conditions of high Mn availability are not known. Although the capacity of this species to accumulate significant amounts of Mn in leaves is known (Motta et al., 2020), the maximum accumulation potential, location of Mn within leaf tissues, phytotoxic damage, and consequent effects on plant growth have not been explored.

Therefore, the present study aims to: (1) investigate possible toxicity effects and the consequences of high Mn concentration on growth and leaf elemental composition; (2) determine the maximum Mn accumulation potential of I. paraguariensis leaves; (3) investigate the location of Mn within leaf tissue; and (4) assess the effect of soil pH increase on Mn accumulation. Results of the present work will aid in understanding Mn uptake dynamics from soil to *I. paraguariensis* leaves and Mn storage in leaves. Additionally, results may have implications within production and manufacturing systems since *I. paraguariensis* is part of the human diet and excessive Mn consumption may impact human health.

5.4 MATERIALS AND METHODS

5.4.1 EXPERIMENTAL DESIGN AND SOIL COLLECTION

The study was conducted in the understory of an Araucarian forest (*Araucaria angustifolia* L.). This predominantly subtropical region has an altitude of 934 m, with a Köppen classification climate of Cfb (Alvares et al., 2013). The average precipitation during the experimental period was 166.5 mm month^{-1} (67.4–301.2 mm month^{-1}) and the average temperature was 21.0 °C (17.3–26.7 °C). The experiment was conducted in a completely randomized design with four replications, in which two soils from different parent materials (basalt and sandstone) were evaluated with and without acidity correction (liming) at Mn application doses of 0, 30, 90, 270, 540, and 1,080 mg Mn Kg^{-1} soil.

Soil was collected from the 0–20 cm layer, homogenized, and sieved to pass a 4 mm mesh. Soil formed from basalt was classified as a Ferralsol and was collected in *Barão de Cotegipe*, *Rio Grande do Sul* state, Brazil (27°33’50.71” S, 52°24’3.83” W). The soil formed under sandstone was classified as a Cambisol and was collected in *São João do Triunfo*, *Paraná* state, Brazil (25°40’45.05” S, 50°18’ 36.04” W).

5.4.2 DETERMINATION OF SOIL CHEMICAL AND PHYSICAL PARAMETERS

Prior to experiment installation, texture was determined by the Bouyoucos hydrometer method using as a dispersant mixture of NaOH (4 g L^{-1}) and $(NaPO_3)_6$ (10 g L^{-1}) (Dane et al., 2002). Soil chemical and physical properties are shown in TABLE 1. After an acidity corrective incubation period, another soil sample was taken and chemical characterization was performed again. In both soil analyses, samples were dried at room temperature and ground to pass a 2 mm mesh sieve to determine the following measures: pH (0.01M $CaCl_2$), potential acidity (H + Al), exchangeable acidity (Al^{3+}, by titration), quantification of exchangeable bases (Ca^{2+}, Mg^{2+} extracted by 1 mol L^{-1} KCl, and K^+ extracted with Mehlich-I), available P (extracted with Mehlich-I), and organic C (wet combustion). Chemical determinations followed the methodologies described by Marques and Motta (2003).

TABLE 1 – CHEMICAL AND PHYSICAL ANALYSES OF BASALT AND SANDSTONE SOILS WITH AND WITHOUT LIME ADDITION PRIOR TO PLANTING. OC = ORGANIC CARBON.

Soil	**pH $CaCl_2$**	**Al^{3+}**	**H+Al**	**Ca^{+2}**	**Mg^{+2}**	**K^+**	**P**	**Mn**	**OC**	**Sand**	**Silt**	**Clay**
		------------ $cmol_c$ dm^{-3} ------------					mg dm^{-3}		g dm^{-3}	---- g kg^{-1} ----		
					No lime							
Sandstone	3.5	5.71	20.4	0.7	0.3	0.24	1.5	41	37.3	400	300	300
Basalt	4.4	1.12	12.1	2.8	2.0	0.23	3.8	228	20.1	50	225	725
					Lime							
Sandstone	5.2	0.00	5.4	10.7	1.7	0.16	9.1	38	37.3	400	300	300
Basalt	5.5	0.00	4.3	9.2	2.9	0.43	7.4	207	20.1	50	225	725

SOURCE: The author (2021).

5.4.3 CORRECTION OF ACIDITY AND Mn DOSES

Based on soil analyses, the amount of lime required to achieve a base saturation of 70% was calculated. Thus, 2.80 g kg^{-1} of $CaCO_3$ and 0.28 g kg^{-1} of MgO (both PA reagents) were added to the basalt soil. For the sandstone soil, 4.88 g $CaCO_3$ and 0.50 g MgO per kg of soil were added. Soils were then homogenized, placed in 5 dm^3 containers and incubated for 21 days while keeping soils moist. After incubation, clonal plants of *I. paraguariensis* BRS BLD Yari cultivar propagated by the mini-cuttings technique (Wendling et al., 2017) were transplanted into each container. At 65 days after transplanting, fertilization with N, P, and K equivalent to 150 mg N, 250 mg P_2O_5 and 200 mg K_2O per kg of soil was performed. For this fertilization, we used 117.15 g of K_2HPO_4 + 46.76 g of KH_2PO_4 + 89.08 g of $(NH_4)_2HPO_4$ + 250.59 g of $(NH_4)_2SO_4$ + 26.06 g of KCl diluted in 4.8 L of deionized water and applied 50 mL of solution to each container.

Addition of Mn was split into three application periods to avoid precipitation of Mn and possible plant mortality at high doses. The application of Mn occurred at 41, 65, and 153 days after transplanting. Thus, it was possible to progressively monitor plant stress due to high Mn availability. The source used was manganese sulfate ($MnSO_4$) diluted in deionized water, and three applications of 0, 10, 30, 90, 180, 360 mg kg^{-1} of soil totaled the initially proposed doses of 0, 30, 90, 270, 540 and 1,080 mg Mn Kg^{-1} of soil.

5.4.4 PLANT MONITORING AND ELEMENTARY QUANTIFICATION

Plants were grown up to 50 days after the last Mn application, which corresponds to 203 days after transplanting. During this period, plants were monitored to diagnose for leaf anomalies indicative of Mn toxicity; symptoms were recorded and characterized in detail. At study termination, plants were cut at the collar region and completely expanded leaves were separated for elemental composition determinations. All plant material was oven dried by forced air circulation at 65 °C until constant mass prior to weight determinations.

Dried fully expanded leaves used to determine elemental composition were ground in a Willey mill to pass a 2 mm mesh sieve. For digestions, 1 g of plant tissue and 10 mL of 3 mol HCl were used and subjected to heat plate digestion at 200 °C for 25 min. After this period, solutions were filtered with 1–2 µm particle retention filter paper, and volume adjusted to 100 mL with deionized water. Elemental quantification of K, Ca, Mg, P, S, Mn, Al, Fe, Zn, B, and Cu was performed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP OES, Varian 720-ES). Certified reference material (GBW–10016 *Camellia sinensis*) was utilized to ensure quality control, and the following recovery values were obtained: K – 107%, Ca – 91%, Mg – 113%, P – 95%, S – 91%, Mn – 104%, Fe – 73%, Zn – 100%, and Cu – 79%.

5.4.5 MICROSCOPY ANALYSIS

Microscopy analysis was performed on plants subjected to Mn doses of 0 and 1,080 mg kg^{-1} (control and maximum dose). After cutting plants, one fully expanded representative leaf from control and maximum dose plants were selected. A section cut from each leaf, resulting in a ~0.5 x 2.0 cm sample that was fixed in the dark (48 h at 4 °C) in 1.5 mL eppendorf microtubes with 1 mL of FAA fixation solution; i.e., 70% ethanol [v/v] + formalin 5% [v/v] + glacial acetic acid 5% [v/v]. After this fixation period, samples were submitted to dehydration by an ethanol series of 80, 90, 95, and 100% (20 min per step). Microanalyses were performed using a SEM

(Vega3 LM, Tescan) equipped with an EDX elemental detector (X-Max^N 80 mm^2, Oxford).

5.4.6 DATA ANALYSIS

Statistical analyzes were performed using R software, version 3.6.0 (R Core Team, 2019). Data was subjected to Shapiro-Wilk and Bartlett tests to verify the assumptions of residual normality ("shapiro.test" functions) and variance homogeneity ("bartlett.test" function), respectively. In cases where assumptions were not met, data were transformed by the optimal Box-Cox power ("boxcox" function of the MASS package). Analysis of variance for each soil was applied according to the completely randomized design with factorial treatments (2 x 6) with four replications, considering doses (6) as quantitative factors and acidity correction (2) as qualitative, and leaf Mn concentration as the response variable. A 95% confidence level was considered for model validation. After validation, regression analysis was applied to determine the response of Mn concentration as a function of the supply of this element to different soils without and with liming ("lm" function). Plant total dry matter results were converted to relative yield, considering the average repetition result, with the zero dose treatment corresponding to 100% yield, and the other doses proportional to this value.

In addition, a Principal Component Analysis (PCA) of elemental composition and total dry matter ("princomp" function) was performed from the correlation matrix of standardized data for mean zero and variance one (transformed by the "decostand" function of Vegan package). The number of Principal Components (PC) used were selected to explain at least 70% of the variance. Plants with and without leaf anomaly were classified into two distinct groups, and from the observation of PCA, a Discriminant Function Analysis (DFA) was applied to discriminate these two groups (MDA package – "lda" function). In the calculation of DFA, we used the original covariance matrix of the data. Precision of the analysis was determined by the confusion matrix. Additionally, the Student's t-test was applied to compare variables used in DFA according to the created groups.

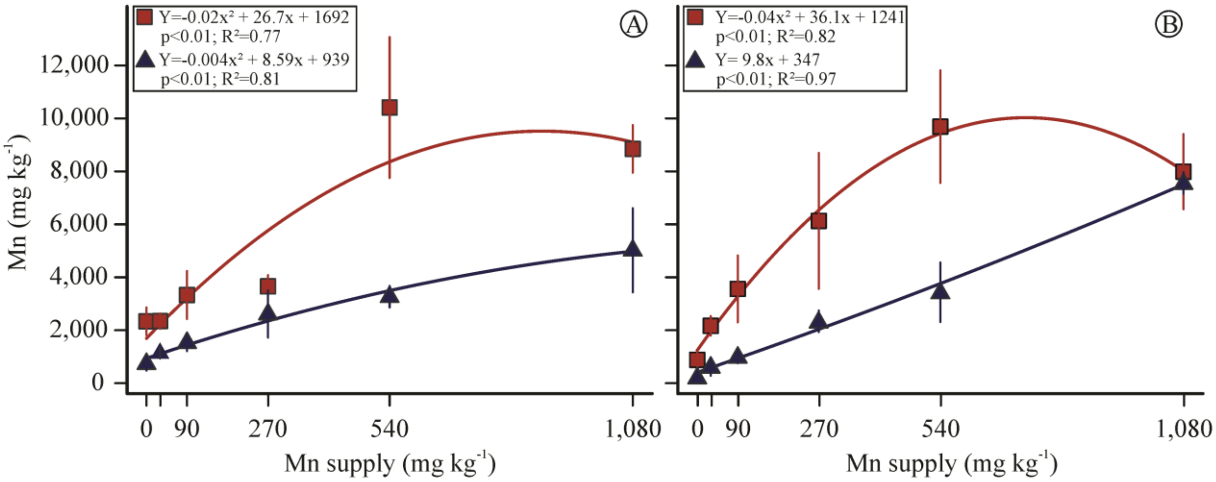
5.5 RESULTS

5.5.1 LEAF Mn CONCENTRATION AND TOXICITY

Concentration of Mn in leaves of *I. paraguariensis* increased with Mn supply (FIGURE 1). The highest Mn concentration in leaves occurred in unlimed soils at the 540 mg kg^{-1} dose, reaching a maximum value of 13,452 mg kg^{-1} for basalt soil and 12,127 mg kg^{-1} for sandstone soil. In limed soils, the respective maximum leaf Mn concentrations of 7,203 mg kg^{-1} and 8,030 mg kg^{-1} for basalt and sandstone soils occurred at the maximum dose.

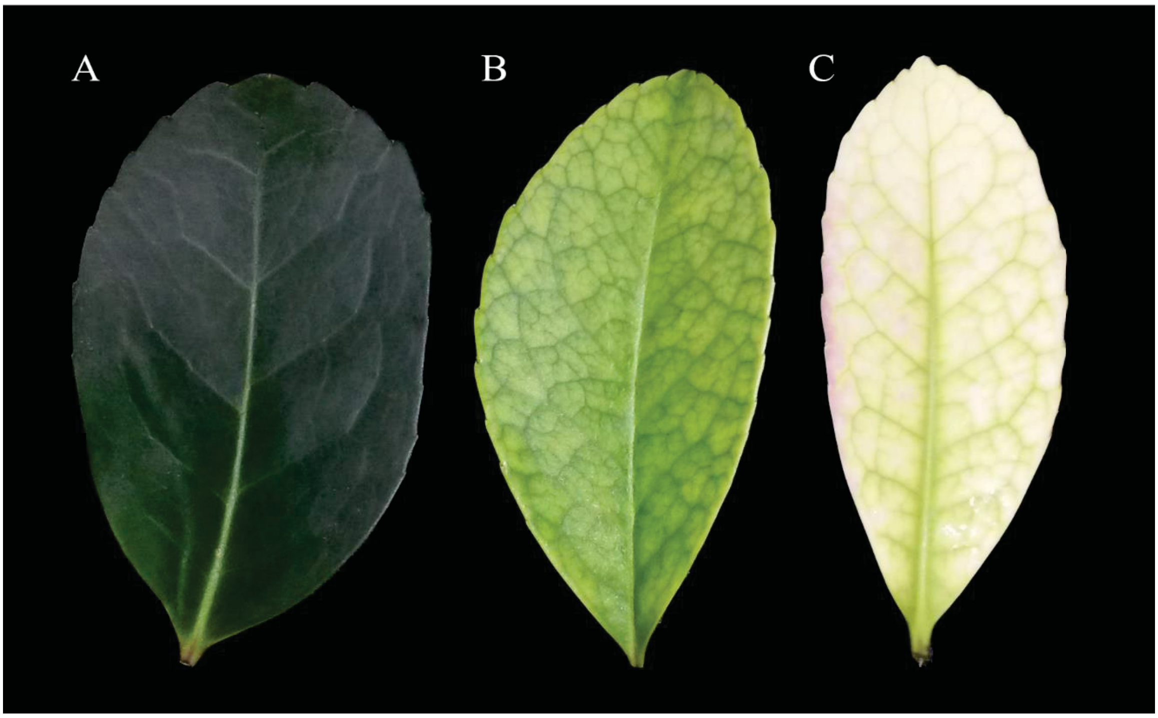
In some treatments, leaves showed toxicity symptoms (FIGURE 2) that was characterized as interveinal chlorosis resulting in leaves without pigmentation in more severe stages (FIGURE 2C). This toxicity symptom started from the second Mn application. Symptom intensity was greatest during initial leaf development, becoming less noticeable with leaf aging, and was barely visible or sometimes absent at the end of the experiment.

FIGURE 1 – Mn CONCENTRATIONS (AVERAGE AND ERROR BARS) IN *Ilex paraguariensis* LEAVES GROWN IN BASALT (A) AND SANDSTONE SOIL (B) WITHOUT LIMING (RED) AND WITH LIMING (BLUE) IN RESPONSE TO Mn SUPPLY (0, 30, 90, 270, 540, AND 1,080 mg kg^{-1}).



SOURCE: The author (2021).

FIGURE 2 – NORMAL *Ilex paraguariensis* LEAF (A) AND LEAVES WITH SYMPTOMS OF INTERVEINAL CHLOROSIS (B AND C) ASSOCIATED WITH INCREASED MN CONCENTRATION.

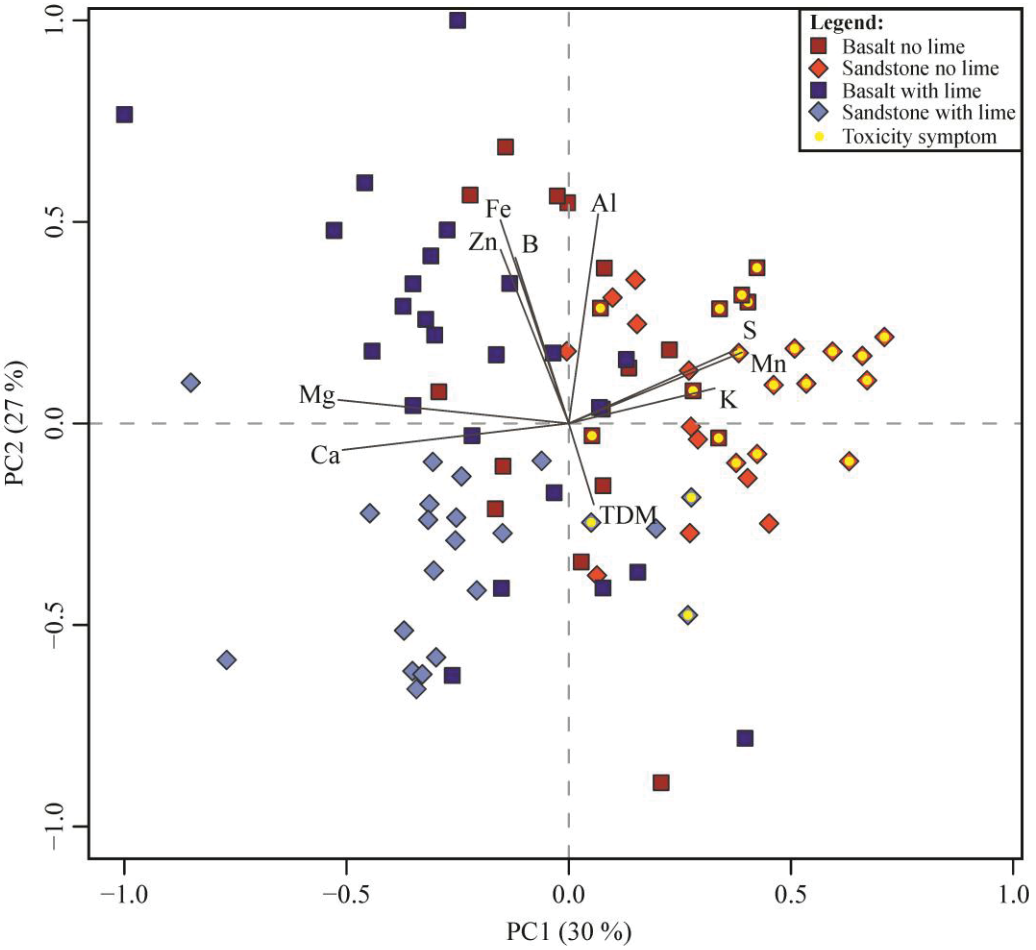


SOURCE: The author (2021).

In unlimed soil, the toxicity symptom was more frequent and occurred at the Mn dose of 360, 540 and 1,080 mg kg^{-1} in the sandstone soil, and at the Mn dose of 540 and 1,080 mg kg^{-1} in the basalt soils. In limed soils, leaf toxicity occurred only at the maximum dose in the sandstone soil and had less intensive chlorosis (FIGURE 2B) than plants grown in unlimed soils. Plants grown in limed basalt soil did not presented toxicity symptoms. As illustrated by PCA, the predominant occurrence of toxicity symptom in plants indicates that this occurred due to excessive Mn accumulation in leaves of *I. paraguariensis* (FIGURE 3). This was confirmed by DFA, which discriminated plants by the presence of toxicity symptom with a 98% accuracy confounded by only one sample from each predetermined group. The main discriminant variables of groups with and without interveinal chlorosis were Mn and Fe leaves concentrations (with negative correlations) based on the discriminant function presented in Eq. (1):

$$D(x) = -0.29Mn[\,] + 0.23Fe[\,] - 0.09Al[\,] + 0.07TDM + 0.04B[\,] \qquad (1)$$

FIGURE 3 – PRINCIPAL COMPONENT ANALYSIS OF LEAF ELEMENTAL COMPOSITION OF *Ilex paraguariensis* GROWN IN BASALT AND SANDSTONE SOILS LIME AND UNLIMED, AT DIFFERENT SUPPLIED MN DOSES (0, 30, 90, 270, 540, AND 1,080 mg kg^{-1}).

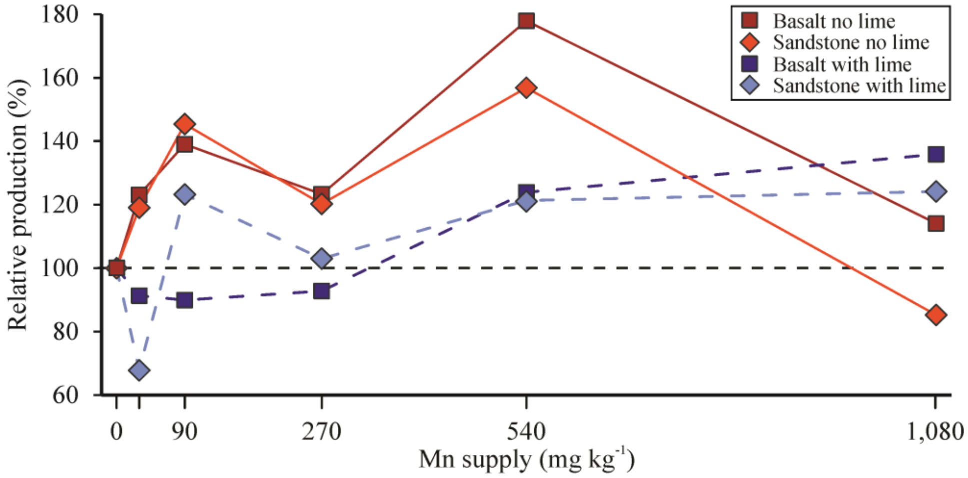


SOURCE: The author (2021).

5.5.2 PLANT GROWTH AND ELEMENTAL COMPOSITION

Increased dose of Mn applied was accompanied by increased plant dry matter yield, with relative dry matter production ~180% and 120% in unlimed and limed basalt soil at the 540 mg kg^{-1} dose, respectively (FIGURE 4). In unlimed soils, an increase in total dry matter production was observed at the lowest Mn doses, with a decrease at the maximum dose; a result that follows the behavior of leaf Mn concentration (FIGURES 1 and 4) that may be reflective of physiological stress by toxicity. In limed soils, the opposite effect was observed, with a decrease in production at the first Mn dose and an increase at the two highest doses.

FIGURE 4. RELATIVE DRY MATTER PRODUCTION OF *Ilex paraguariensis* GROWN IN BASALT AND SANDSTONE SOIL WITHOUT LIMING AND WITH LIMING IN RESPONSE TO MN SUPPLY (0, 30, 90, 270, 540, AND 1,080 mg kg^{-1}).



SOURCE: The author (2021).

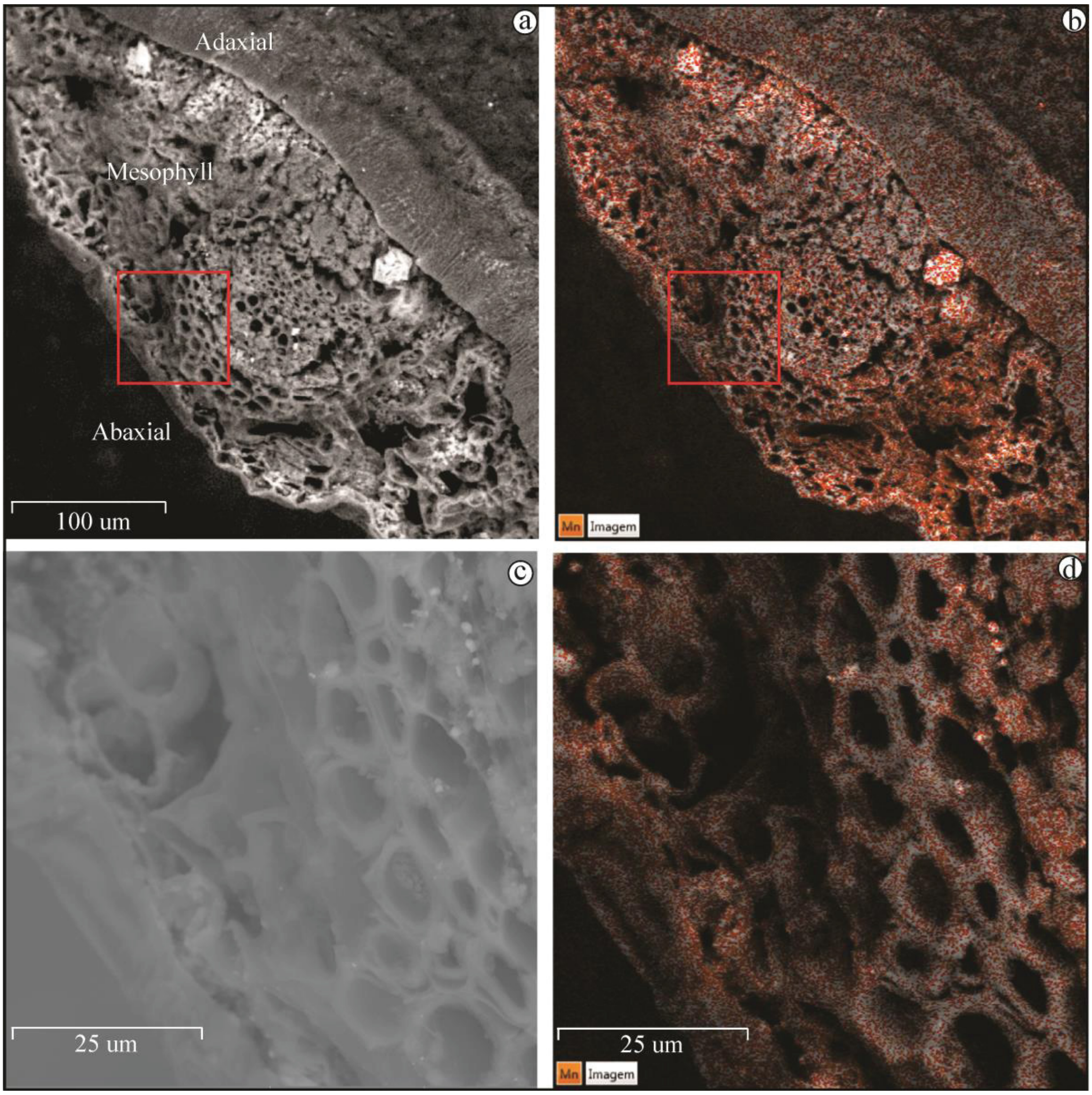
LEGEND: Horizontal dashed line represents the witness (100% relative yield).

Increases in Mn leaf elemental concentration was accompanied by increased in S concentration. This Mn increase did not affect concentrations of any other evaluated element (TABLE 2). Three main Principal Components (PC) can explain 70% of data variation for leaf elemental composition and total dry matter (FIGURE 3 and TABLE S1). PC1 showed positive correlation between Mn, S, and K, and these elements were negatively correlated to concentrations of Ca and Mg. FIGURE 3 clearly shows the presence of higher Mn concentration in unlimed soils. PC2 represented the positive relationships between Fe, Al, Zn, and B (TABLE S1). The highest values of these elements were associated with plants grown in soil with Mn doses of 30, 90, 270, and 540 mg kg^{-1} (TABLE 2). PC3 reflects total dry matter production, which was positively correlated with Mn and S concentrations and negatively correlated with K (TABLE S1).

5.5.3 DISTRIBUTION OF ELEMENTS IN YERBA MATE LEAVES

Low concentration in the control treatment prevented detection of Mn in yerba mate leaf samples. SEM–EDS evaluations of the maximum dose treatment revealed no presence of Mn precipitates in leaves. This element was concentrated in the mesophyll region, primarily in vascular bundles (FIGURE 5).

FIGURE 5 – MEV-EDS PHOTOMICROGRAPHS SHOWING MN DISTRIBUTION IN *Ilex paraguariensis* LEAVES FROM THE MAXIMUM DOSE TREATMENT (A AND B) AND ZOOMED AREAS OUTLINED IN RED (C AND D).



SOURCE: The author (2021).

5.6 DISCUSSION

5.6.1 ACCUMULATION OF Mn IN LEAVES

The maximum observed Mn concentration (13,452 mg kg^{-1}) (FIGURE 1) was approximately a third higher than the previous maximum reported in the literature for *I. paraguariensis* leaves (Motta et al., 2020). Thus, under experimental conditions this species displays characteristics of Mn hyperaccumulating plants by exceeding Mn concentrations of 10,000 mg kg^{-1} (Baker and Brooks, 1989). The maximum concentrations of Mn obtained for both soils without liming were similar. Additionally, for both soils at the highest dose (1,080 mg kg^{-1}), leaf Mn concentration decreased in relation to the previous dose (540 mg kg^{-1}); this suggests that these accumulation values are the maximum for this species. Caldeira et al. (2006) found Mn concentrations above 1,000 mg kg^{-1} in *Ilex dumosa* (Reissek) leaves under native conditions, indicating that other species of the genus *Ilex* may accumulate high amounts of Mn in leaves. Given that the critical concentration for the definition of Mn hyperaccumulating species (>10,000 mg kg^{-1}) proposed by Baker and Brooks (1989) has been widely used (Reeves et al., 2018; Van der Ent et al., 2019), *I. paraguariensis* could be considered a Mn hyperaccumulator species under our study conditions. However, other standards has been proposed, such as bioconcentration factor (>1; but often >50), shoot-to-root factor, and the genetic approach (Van der Ent et al., 2013). Motta et al. (2020) reported a bioconcentration factor (plant:soil ratio) between 12 and 14 for *I. paraguariensis* plants with leaf Mn concentrations of 9,401 ± 1,591 mg kg^{-1}; considering the study as a whole (30 sites), the bioconcentration factor ranged from 0.9 to 32.

For healthy growth, plants resistant to high Mn concentration employ mechanisms to maintain this element in non-toxic forms. The most common strategies being associations with the endoplasmic reticulum and Golgi complex, storage in vacuoles and epidermal cells, and Mn chelate formations (Shao et al., 2017). Since toxicity symptoms tend to disappear with leaf aging, *I. paraguariensis* possibly uses some of these mechanisms to maintain Mn in a non-toxic form, which allows mature leaves with Mn concentrations above 10,000 mg kg^{-1} to not manifest visual symptoms in mature leaves. Although present in high concentration, no high Mn precipitates were observed in foliar tissues (FIGURE 5), suggesting that this element was homogeneously distributed throughout the mesophyll. As indicated by Junior et al. (2008), Mn may be allocated to negative cell wall charges, cytoplasm, and vacuoles. Thus, distribution in mesophyll indicates Mn was adsorbed to charges of cell walls or existed as free forms in the vascular bundle.

Motta et al. (2020) showed that parent material of soil plays a key role in influencing Mn concentration in *I. paraguariensis* leaves and that concentration in basalt soils is about twice as large as sedimentary and rhyolite/rhyodacite soils. In our study, this effect was clear in unlimed soils without Mn supply, where leaf Mn concentrations were 2,336 ± 457 and 884 ± 295 mg kg^{-1} in basalt and sandstone soil, respectively (FIGURE. 1). In this context, Barbosa et al. (2018) noted an influence of soil type on leaf Mn concentrations related to crystalline and low crystallinity forms of this nutrient in soil.

Although leaf Mn concentrations followed the amount of soil Mn availability, two other factors can influence this difference: in poorly drained areas (e.g., low slopes or wet slopes), higher Mn availability can be expected (Zengin, 2013); climate variations could increase availability and accumulation of Mn during rainy seasons as has been observed in pastures (Senger et al., 1997; Siman et al., 1974). This implies that a large variation in leaf Mn concentration could occur between plants cultivated within the same location depending on climatic variation during the year and position in regard to landscape relief.

On the other hand, increased pH decreased leaf Mn concentration (FIGURE 1), corroborating results of Toppel et al. (2018) who found a relationship between Mn concentrations in native plants of *I. paraguariensis* and soil pH. This indicates that changing soil pH is the most efficient way to reduce Mn concentration in *I. paraguariensis* leaves, and consequently reduce Mn concentration in the raw material used for infusion preparations. As initially reported, this is an important subject since *I. paraguariensis* mate drinks are consumed by a large population (adolescents, adults, and the elderly) and high Mn consumption can be unhealthy (Leite et al., 2014). Almost half of the Mn present in *I. paraguariensis* leaves is known to be soluble in hot water (Barbosa et al., 2015). Therefore, the ecotoxicology of plants can have direct or indirect effects on consumers. However, a better understanding of the amount of Mn that is effectively absorbed by the body is required. On the other hand, there is great potential for use of these Mn-enriched leaves for nutritional supply of both humans (those needing Mn supplements) and breeding animals.

5.6.2 PLANT GROWTH AND ELEMENTAL INTERACTION

In comparison to typical cultivated plants, pH increase was accompanied by a reduction in *I. paraguariensis* dry matter accumulation (FIGURE 4). This was also observed by Santin et al. (2013) under field conditions. This effect on growth can be linked to the reduction in leaf Mn concentration, since this element was the only one affected in our study. Growth promoted by increased Mn concentration can be associated with distinct physiological effects. First, Mn^{2+} has a similar ionic radius compared to Mg^{2+} and Ca^{2+} (0.075 nm, 0.065 nm and 0.099 nm, respectively) and is able to replace these elements in their functions (Broadley et al., 2012). Another characteristic is that Mn is responsible for cell elongation, and directly affects root growth and subsequent plant growth (Sadana et al., 2002). Finally, high Mn concentrations promote the degradation of Indole-3-Acetic-Acid (IAA) due to increased oxidase and polyphenoloxidase activity (Fecht-Christoffers et al., 2007). Decreased IAA promotes loss of apical dominance and the formation of auxiliary shoots (Bañados et al., 2009), which can contribute to increased leaf emergence and overall increases in plant dry matter. However, it is important to highlight that high pH can harm *I. paraguariensis* due to non-nutritional effects, such as increase in root diseases (Poletto et al., 2011). Plants with toxicity symptom exhibited the highest Mn concentrations (FIGURES 1 and 2). However, Mn toxicity is often confused with deficiencies of Fe, Ca, Mg, or Zn (Zanão Júnior et al., 2010). Thus, occurrence of interveinal chlorosis was attributed to excess Mn and associated with lower Fe concentrations. The average concentration of Mn in leaves with interveinal chlorosis was more than three times higher than leaves displaying no toxicity symptoms, while Fe was approximately 20% lower (TABLE S2). As a consequence, chlorophyll concentration in plants may have decreased due to Fe deficiency induced by excess Mn (Silva et al., 2017). Huang et al. (2016) observed a similar situation in sugarcane with Mn toxicity, where the average amount of total Fe was slightly lower in plants with chlorosis; however, active Fe corresponded to 30% of amounts found in normal plants (i.e., although plants had sufficient Fe, excess Mn inhibited normal functions). This lack of physiologically active Fe is a side effect of Mn toxicity that blocks the synthesis of chlorophyll and results in chlorosis (Subrahmanyam and Rathore, 2000; Huang et al., 2016). In addition, excess Mn can replace Mg in the chlorophyll molecule, causing damage that also results in chlorosis (Clairmont et al., 1986). However, this toxicity symptom (FIGURE 2) is probably due to the application of a high dose of Mn to the soil; under these conditions, there are no records of symptoms in leaves with high concentrations of Mn (Motta et al., 2020).

The lower occurrence of interveinal chlorosis in plants cultivated in limed soils is due to increased soil pH along with the addition of Ca and Mg and is very clear in the PCA (FIGURE 3). Liming effects are not restricted to soil pH elevation, but are also attributable to increasing Ca amounts near roots, which reduces Mn absorption capacity due to competition for absorption sites (Fernando and Lynch, 2015). In addition, Mg supplied by lime also contributes to reduction of Mn toxicity symptoms in plants (Davis, 1996).

Concentrations of Zn, Fe, and Mg in leaves with interveinal chlorosis were slightly lower than leaves without symptoms (Table 2), while Ca concentration was ~50% lower. However, the literature indicates that Mn toxicity associated with low Ca concentrations causes leaf apex deformation (Broadley et al., 2012), but this was not observed in the present study. Zinc deficiency is associated with reduced growth, while Mg deficiency is symptomatic in old leaves (Broadley et al., 2012). These results reinforce the fact that toxicity is more related to imbalance with Fe than with other nutrients. Regarding nutritional composition (Table 2), an increase in S concentration was expected since the source of Mn used was $MnSO_4$.

There are also reports of mycorrhiza associations with yerba mate roots, but the effect of this association on elemental composition is not known (Bergottini et al., 2017; Silvana et al., 2020). This highlights a knowledge gap that is worthy of future investigation.

5.7 CONCLUSION

The maximum concentrations of Mn in *I. paraguariensis* leaves were 13,452 mg kg^{-1} for basalt soil and 12,127 mg kg^{-1} for sandstone soil, indicating that this species may be a Mn hyperaccumulator, which was facultative since this effect was soil-dependent. This is reinforced by the effect of Mn on the physiology of the plant, which showed a positive growth response and had no detrimental effect on nutritional status. Additionally, since leaves show symptoms of phytotoxicity during initial stages of leaf development (likely associated with Fe deficiency) that disappeared with further leaf development, Mn was probably transformed to non-toxic forms with leaf aging. Considering that products derived from *I. paraguariensis* are part of the human diet, increasing pH by lime application can drastically reduce concentrations of Mn in leaves, thereby reducing Mn consumed in infusion products. However, increasing soil pH can decrease *I. paraguariensis* growth. Thus, it is necessary to identify optimal lime doses that decreases Mn in leaves without negatively impacting *I. paraguariensis* yield.

5.8 REFERENCES

Althaus, D., Gianello, C., Tedesco, M. J., Silva, K. J. D., Bissani, C. A. & Felisberto, R. (2018). Natural fertility and metals contents in soils of Rio Grande Do Sul (Brazil). *Revista Brasileira de Ciência do Solo*, p. 42.

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M. & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22(6), 711–728.

Baker, A. J., & Brooks, R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. *Biorecovery*, 1(2), 81-126.

Bañados, M. P., Ibáñez, F., & Toso, A. M. (2008, July). Manganese toxicity induces abnormal shoot growth in'O'Neal'blueberry. In *IX International Vaccinium Symposium 810* (pp. 509-512).

Barbosa, J. Z., Motta, A. C., Consalter, R., Poggere, G. C., Santin, D., & Wendling, I. (2018). Plant growth, nutrients and potentially toxic elements in leaves of yerba mate clones in response to phosphorus in acid soils. *Anais da Academia Brasileira de Ciências*, 90(1), 557-571.

Barbosa, J. Z., Motta, A. C. V., dos Reis, A. R., Corrêa, R. S., & Prior, S. A. (2020). Spatial distribution of structural elements in leaves of *Ilex paraguariensis*: physiological and ecological implications. *Trees*, 34(1), 101-110.

Barbosa, J. Z., Zambon, L. M., Motta, A. C. V. & Wendling, I. (2015). Composition, hot-water solubility of elements and nutritional value of fruits and leaves of yerba-mate. *Ciência e Agrotecnologia,* 39(6), 593–603.

Bergottini, V. M., Hervé, V., Sosa, D. A., Otegui, M. B., Zapata, P. D., & Junier, P. (2017). Exploring the diversity of the root-associated microbiome of *Ilex paraguariensis* St. Hil.(Yerba Mate). *Applied Soil Ecology*, 109, 23-31.

Broadley, M., Brown, P., Cakmak, I., Rengel, Z., & Zhao, F. (2012). Function of nutrients: micronutrients. *In Marschner's mineral nutrition of higher plants* (pp. 191-248). Academic Press.

Caldeira, M. V. W., Watzlawick, L. F., Soares, R. V., & Valério, A. F. (2006). Teores de micronutrientes em espécies arbóreas da floresta ombrófila mista Montana-General Carneiro/PR. *Ambiência*, 2(1), 29-50.

Clairmont, K. B., Hagar, W. G., & Davis, E. A. (1986). Manganese toxicity to chlorophyll synthesis in tobacco callus. *Plant physiology*, 80(1), 291-293.

Dane, J. H., Topp, C. G. & Campbell, G. S. (2002). Particle size analysis. In *Methods of soil analysis. Part 4: Physical methods*. Soil Science Society of America Book Series, 5, 255-293.

Davis, J. G. (1996). Soil pH and magnesium effects on manganese toxicity in peanuts. *Journal of plant nutrition*, 19(3-4), 535-550.

Fecht-Christoffers, M. M., Maier, P., Iwasaki, K., Braun, H. P., & Horst, W. J. (2007). The role of the leaf apoplast in manganese toxicity and tolerance in cowpea (Vigna unguiculata L. Walp). In *The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions* (pp. 307-321). Springer, Dordrecht.

Fernando, D. R., & Lynch, J. P. (2015). Manganese phytotoxicity: new light on an old problem. *Annals of botany*, 116(3), 313-319.

Huang, Y. L., Yang, S., Long, G. X., Zhao, Z. K., Li, X. F., Gu, M. H., 2016. Manganese toxicity in sugarcane plantlets grown on acidic soils of southern China. *PLoS One,* 11 (3), e0148956.

Junior, J. L., Moraes, M. F., Cabral, C. P. & Malavolta, E. (2008). Influência genotípica na absorção e na toxidez de manganês em soja. *Revista Brasileira de Ciências do Solo*, 32 (1), 173–181.

Kabata-Pendias, A. (2010). Trace elements in soils and plants, 4th ed. CRC Press, Pulawy, Poland.

Leite, F. P., Novais, R. F., Silva, I. R., Barros, N. F., Neves, J. C. L., Medeiros, A. G. B., Ventrella, M. C., Villani, E. M. A. (2014). Manganese accumulation and its relation to eucalyptus shoot blight in the Vale do Rio Doce. *Revista Brasileira de Ciências do Solo,* 38(1), 193–204.

Marques, R. & Motta, A. C. V. (2003). Análise química do solo para fins de fertilidade. In: Lima, M.R.de. (Ed.), *Manual de diagnóstico da fertilidade e manejo dos solos agrícolas, 2nd ed.* Departamento de Solos e Engenharia Agrícola, Curitiba, pp. 81–102.

Millaleo, R., Reyes-Diaz, M., Ivanov, A. G., Mora, M. L. & Alberdi, M. (2010). Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition*, 10 (4), 470–481.

Motta, A. C. V., Barbosa, J. Z., Magri, E., Pedreira, G. Q., Santin, D., Prior, S. A., Consalter, R., Young, S. D., Broadley, M. R. & Benedetti, E. L. (2020). Elemental composition of yerba mate (*Ilex paraguariensis* A. St.-Hil.) under low input systems of southern Brazil. *Science of the Total Environment,* 736, 139637.

Oliva, E. V., Reissmann, C. B., Gaiad, S., Oliveira, E. B. & Sturion, J. A., 2014. Composição nutricional de procedências e progênies de erva-mate (*Ilex paraguariensis* St. Hil.) cultivadas em latossolo vermelho distrófico. *Ciência Florestal*, 24(4), 793–805.

Poletto, I., Muniz, M. F. B., Ceconi, D. E. & Blume, E. (2011). Influência da aplicação de NPK e calcário sobre o crescimento da erva-mate, severidade da podridão-de-raízes e população fúngica do solo. *Ciência Florestal*, 21 (3), 429–444.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

Reeves, R. D., Van Der Ent, A. & Baker, A. J. (2018). Global distribution and ecology of hyperaccumulator plants. In: *Van Der Ent,* A., Echevarria, G., Baker, A.J.M., Morel, J.L. (Ed.), Agromining: Farming for Metals. Springer, pp. 75–92.

Reissmann, C. B. & Carneiro, C. (2004). Crescimento e composição química de erva-mate (*Ilex paraguariensis* St. Hil.), transcorridos oito anos de calagem. *Floresta,* 34(3), 381–386.

Reissmann, C. B., Radomski, M. I. & Quadros, R. M. B. (1999). Chemical composition of *Ilex paraguariensis* St. Hil. under different management conditions in seven localities of Paraná State. *Brazilian Archives of Biology and Technology,* 42(2), 187–194.

Sadana, U. S., Kusum, L. & Claassen, N. (2002). Manganese efficiency of wheat cultivars as related to root growth and internal manganese requirement. *Journal of Plant Nutrition*. 25, 2677–2688.

Santin, D., Benedetti, E. L., Kaseker, J. F., Bastos, M. C., Reissmann, C. B., Wendling, I. & Barros, N. F. D. (2013)b. Nutrição e crescimento da erva-mate submetida à calagem. *Ciência Florestal*, 23(1), 55-66.

Schmidt, S. B., Jensen, P. E. & Husted, S. (2016). Manganese deficiency in plants: the impact on photosystem II. *Trends Plant Science*, 21(7), 622–632.

Senger, C. C. D., Sanchez, L. M. B., Pires, M. B. G. & Kaminski, J. (1997). Teores minerais em pastagens do Rio Grande do Sul. II. Sódio, enxofre, zinco, cobre, ferro e manganês. *Pesquisa Agropecuária Brasileira*, 32(1), 101-108.

Shao, J. F., Yamaji, N., Shen, R. F. & Ma, J. F. (2017). The key to Mn homeostasis in plants: regulation of Mn transporters. *Trends Plant Science*, 22(3), 215–224.

Silva, D. M. da, SOURCE, N. dos. S. da, Souza, K. R. D. de, Rodrigues-Brandão, I., Libeck, I. T. & Alves, J. D. (2017). Relationship between manganese toxicity and waterlogging tolerance in *Zea mays* L. cv. Saracura. *Acta Scientiarum. Agronomy*. 39(1), 75–82.

Silvana, V. M., Carlos, F. J., Lucía, A. C., Natalia, A. & Marta, C. (2020). Colonization dynamics of arbuscular mycorrhizal fungi (AMF) in *Ilex paraguariensis* crops: Seasonality and influence of management practices. *Journal of King Saud University – Science*, 32(1), 183-188.

Siman, A., Cradock, F. W. & Hudson, A. W. (1974). The development of manganese toxicity in pasture legumes under extreme climatic conditions. *Plant Soil,* 41(1), 129–140.

Subrahmanyam, D. & Rathore, V. S. (2000). Influence of manganese toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings. *Photosynthetica*, 38(3), 449–453.

Toppel, F. V., Junior, A. M., Motta, A. C. V., Frigo, C., Magri, E. & Barbosa, J. Z. (2018). Soil chemical attributes and their influence on elemental composition of yerba mate leaves. *Floresta*, 48(3), 425-434.

Valduga, A. T., Gonçalves, I. L., Magri, E. & Finzer, J. R. D. (2019). Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. *Food Research International*. Int. 120, 478–503.

Van der Ent, A., Baker, A. J., Reeves, R. D., Pollard, A. J. & Schat, H. (2013). Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil. 362(1), 319-334.

Van der Ent, A., Ocenar, A., Tisserand, R., Sugau, J. B., Echevarria, G., Erskine, P. D. (2019). Herbarium X-ray fluorescence screening for nickel, cobalt and manganese hyperaccumulator plants in the flora of Sabah (Malaysia, Borneo Island). *Journal of Geochemical Exploration*, 202, 49-58.

Wendling, I., Santin, D., Nagaoka, R. & Sturion, J. A. (2017). BRS BLD Aupaba e BRS BLD Yari: cultivares clonais de erva-mate para produção de massa foliar de sabor suave. *Embrapa Florestas-Comunicado Técnico* (INFOTECA-E).

Xue, S. G., Chen, Y. X., Reeves, R. D., Baker, A. J., Lin, Q., Fernando, D. R. (2004). Manganese uptake and accumulation by the hyperaccumulator plant *Phytolacca acinosa* Roxb. (Phytolaccaceae). *Environmental Pollution*, 131 (3), 393–399.

Zanão Júnior, L. A., SOURCEs, R. L. F., Neves, J. C. L., Korndörfer, G. H. & Ávila, V. T. (2010). Rice grown in nutrient solution with doses of manganese and silicon. *Revista Brasileira de Ciências do Solo*, 34 (5), 1629–1639.

Zengin, F. (2013). Biochemical and physiological effect of excess manganese (Mn) in bean (*Phaseolus vulgaris* L. cv. Strike). *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, Sci. 83 (4), 651–657.

Zhao, H., Wu, L., Chai, T., Zhang, Y., Tan, J. & Ma, S. (2012). The effects of copper, manganese and zinc on plant growth and elemental accumulation in the manganese-hyperaccumulator *Phytolacca americana*. *Journal of Plant Physiology*. 169 (13), 1243–1252.

5.9 SUPPLEMENTARY MATERIAL

TABLE S1 – RESULTS OF PRINCIPAL COMPONENT ANALYSIS OF ELEMENTAL COMPOSITION OF LEAVES AND TOTAL DRY MATTER FROM *Ilex paraguariensis* GROWN UNDER DIFFERENT Mn DOSES (0, 30, 90, 270, 540, AND 1,080 mg kg^{-1}) IN BASALT AND SANDSTONE SOIL WITH AND WITHOUT LIMING.

Variable	**CP1**		**CP2**		**CP3**	
	Eigenvector	σ^2 (%)	Eigenvector	σ^2 (%)	Eigenvector	σ^2 (%)
Mn	0.39	15.2	0.18	3.1	0.48	22.6
K	0.33	10.7	0.09	0.8	-0.45	20.2
Ca	-0.51	25.8	-0.07	0.5	0.22	5.0
Mg	-0.52	26.9	0.06	0.3	0.12	1.4
Fe	-0.16	2.5	0.50	25.4	0.02	0.0
Al	0.06	0.4	0.52	27.0	-0.02	0.0
Zn	-0.16	2.4	0.43	18.5	-0.03	0.1
B	-0.12	1.5	0.41	16.9	0.11	1.2
S	0.38	14.2	0.19	3.5	0.30	9.2
TDM	0.06	0.3	-0.20	4.0	0.63	40.2
Eigenvalues	1.73		1.63		1.15	
% explained	30		27		13	
% accumulated	30		57		70	

SOURCE: The author (2021).

LEGEND: TDM= Total dry matter.

TABLE S2 – AVERAGE LEAF ELEMENTAL COMPOSITION OF *Ilex paraguariensis*, WITH AND WITHOUT INTERVEINAL CHLOROSIS, FROM PLANTS GROWN UNDER DIFFERENT Mn DOSES (0, 30, 90, 270, 540, AND 1,080 mg kg^{-1}) IN BASALT AND SANDSTONE SOIL WITH AND WITHOUT LIMING.

Symptom	**K**	**Ca**	**Mg**	**P**	**S**	**Mn**	**Al**	**Fe**	**Zn**	**B**	**Cu**	**TDM**
	------------- g kg^{-1} -------------					---------------- mg kg^{-1} ----------------						g plant^{-1}
Presence	12.8	3.9^{**}	3.1^{**}	1.07	1.04^{**}	$8{,}170^{**}$	166	100^{*}	106	13.0	3.3	11.6
Absence	12.4	6.5	4.5	1.26	0.52	2,252	155	123	112	13.5	2.9	11.0

SOURCE: The author (2021).

LEGEND: TDM= Total dry matter.

6 GENERAL CONCLUSION

The presence of Cd and Pb, both in the soil and in the leaves of yerba mate occurs naturally, showing that the current legislation that regulates the limits of these elements in South America is understated. In addition, tea laws in other parts of the world adopt much higher values, which take into account what is naturally present in the plants, considering that the daily intake of Cd and Pb in infusion drinks is low. These results suggest that the current MERCOSUR legislation regarding the maximum limits for Cd and Pb in yerba mate products for infusion needs to be revised.

There are a number of factors that infer the elemental composition of the yerba mate leaves, and consequently the quality of the product generated from them. Of these, the most significant include: the association between leaf B and temperature; the inverse relationship between leaf Mn and soil pH; the leaf N with altitude and soil organic matter and the cropping system (agroforestry and monocultures) with leaf P and Mn contents.

Other results showed that the use of glyphosate to control unwanted plants may impair the initial development of yerba mate, especially in soils lacking P. Additionally, its use can increase the elemental composition of yerba mate leaves, with variations according to the cultivar and the soil in which the plant grows. Overall, the increases observed are not a cause for concern to producers and consumers.

Yerba mate has a marked capacity for Mn accumulation in its leaves without damaging growth. It is considered as a hyperaccumulator of Mn. The uptake and accumulation of Mn by yerba mate is controlled by soil pH and soil source material, which directly affect the amount and availability of Mn.

In conclusion, the current study analyzed the presence of Cd and Pb in the leaves of yerba mate; investigated the edaphoclimatic and management dependencies on the elemental composition of yerba mate; qualified the changes in growth and elemental composition caused by the use of glyphosate; and, finally, proved the hyper-accumulative capacity of Mn in this species of yerba mate.

REFERENCES

Alexander, P., Alloway, B. & Dourado, A. (2006). Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables. *Environment Pollution*, 144 (3), 736–745.

Althaus, D., Gianello, C., Tedesco, M. J., Silva, K. J. D., Bissani, C. A. & Felisberto, R. (2018). Natural fertility and metals contents in soils of Rio Grande Do Sul (Brazil). *Revista Brasileira de Ciência do Solo*, p. 42.

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M. & Sparovek, G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22(6), 711–728.

Argüello, D., Chavez, E., Lauryssen, F., Vanderschueren, R., Smolders, E., & Montalvo, D. (2019). Soil properties and agronomic factors affecting cadmium concentrations in cacao beans: A nationwide survey in Ecuador. *Science of the total Environment*, 649, 120-127.

AVRDC. (1990). Vegetable Production Training Manual. Tainan: Asian Vegetable Research and Development Center. 447p.

Bailey, W. A., Poston, D. H., Wilson, H. P. & Hines, T. E. (2002). Glyphosate interactions with manganese. *Weed Technology*. 16(4), 792-799.

Baker, A. J., & Brooks, R. (1989). Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. *Biorecovery*, 1(2), 81-126.

Bañados, M. P., Ibáñez, F., & Toso, A. M. (2008, July). Manganese toxicity induces abnormal shoot growth in'O'Neal'blueberry. In *IX International Vaccinium Symposium 810* (pp. 509-512).

Barbosa, E. A., Silva, I. M. D., França, A. C., Silva, E. D. B. & Matos, C. D. C. D. (2020a). Evaluation of leaf and root absorptions of glyphosate in the growth of coffee plants. *Arquivos do Instituto Biológico*, 87, 1-8.

Barbosa, J. Z., dos Santos Domingues, C. R., Poggere, G. C., Motta, A. C. V., Dos Reis, A. R., de Moraes, M. F., & Prior, S. A. (2019). Elemental composition and nutritional value of Araucaria angustifolia seeds from subtropical Brazil. *Journal of food science and technology*, 56(2), 1073-1077.

Barbosa, J. Z., Motta, A. C. V., dos Reis, A. R., Corrêa, R. S., & Prior, S. A. (2020). Spatial distribution of structural elements in leaves of *Ilex paraguariensis*: physiological and ecological implications. *Trees*, 34(1), 101-110.

Barbosa, J. Z., Motta, A. C., Consalter, R., Poggere, G. C., Santin, D., & Wendling, I. (2018). Plant growth, nutrients and potentially toxic elements in leaves of yerba mate clones in response to phosphorus in acid soils. *Anais da Academia Brasileira de Ciências*, 90(1), 557-571.

Barbosa, J. Z., Zambon, L. M., Motta, A. C. V. & Wendling, I. (2015). Composition, hot-water solubility of elements and nutritional value of fruits and leaves of yerba-mate. *Ciência e Agrotecnologia,* 39(6), 593–603.

Bayer, C., Lovato, T., Dieckow, J., Zanatta, J. A., & Mielniczuk, J. (2006). A method for estimating coefficients of soil organic matter dynamics based on long-term experiments. *Soil and Tillage Research*, 91(1-2), 217-226.

Beckie, H. J., Flower, K. C. & Ashworth, M. B. (2020). Farming without Glyphosate? Plants, 9(1), 96.

Bellaloui, N., Reddy, K. N., Zablotowicz, R. M., Abbas, H. K. & Abel, C. A. (2009). Effects of glyphosate application on seed iron and root ferric (III) reductase in soybean cultivars. *Journal of Agricultural and Food Chemistry*, 57, 9569-9574.

Bergottini, V. M., Hervé, V., Sosa, D. A., Otegui, M. B., Zapata, P. D., & Junier, P. (2017). Exploring the diversity of the root-associated microbiome of *Ilex paraguariensis* St. Hil.(Yerba Mate). *Applied Soil Ecology*, 109, 23-31.

Bewick, V., Cheek, L., & Ball, J. (2004). Statistics review 10: further nonparametric methods. *Critical care*, 8(3), 1-4.

Boskabady, M., Marefati, N., Farkhondeh, T., Shakeri, F., Farshbaf, A., & Boskabady, M. H. (2018). The effect of environmental lead exposure on human health and the contribution of inflammatory mechanisms, a review. *Environment international*, 120, 404-420.

Boskabady, M., Marefati, N., Farkhondeh, T., Shakeri, F., Farshbaf, A. & Boskabady, M. H. (2018). The effect of environmental lead exposure on human health and the contribution of inflammatory mechanisms, a review. *Environment international*, 120, 404-420.

Bott, S., Tesfamariam, T., Kania, A., Eman, B., Aslan, N., Romheld, V. & Neumann, G. (2011). Phytotoxicity of glyphosate soil residues re-mobilised by phosphate fertilization. *Plant Soil*, 342:249–263

Bracesco, N., Sanchez, A. G., Contreras, V., Menini, T., & Gugliucci, A. (2011). Recent advances on *Ilex paraguariensis* research: minireview. *Journal of ethnopharmacology*, 136(3), 378-384.

Brasil. (2013). Dispõe sobre o Regulamento Técnico MERCOSUL sobre Limites Máximos de Contaminantes Inorgânicos em Alimento (Resolução-RDC nº 42, de 29 de agosto de 2013). *Diário Oficial [da] República Federativa do Brasil.*

Broadley, M., Brown, P., Cakmak, I., Rengel, Z., & Zhao, F. (2012). Function of nutrients: micronutrients. *In Marschner's mineral nutrition of higher plants* (pp. 191-248). Academic Press.

Bugang, W., & Woolsey, M. (2010). National food safety standardmaximum levels of contaminants in food. *People's Republic of china. Gain Report Number-CH10000.*

Cakmak, I., Yazici, A., Tutus, Y. & Ozturk, L. (2009). Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. *European Journal of Agronomy*, 31(3), 114-119.

Caldeira, M. V. W., Watzlawick, L. F., Soares, R. V., & Valério, A. F. (2006). Teores de micronutrientes em espécies arbóreas da floresta ombrófila mista Montana-General Carneiro/PR. *Ambiência*, 2(1), 29-50.

Cardozo-Junior, E. L. & Morand, C. (2016). Interest of mate (Ilex paraguariensis A. St.-Hil.) as a new natural functional food to preserve human cardiovascular health – A review. *Journal of Functional Foods*, 21, 440-454.

Carvalho, F. P., Souza, B. P., França, A. C., Ferreira, E. A., Franco, M. H. R., Kasuya, M. C. M. & Ferreira, F. A. (2014). Glyphosate drift affects arbuscular mycorrhizal association in coffee. *Planta Daninha*, 32(4), 783-789.

Castanheira, D. T., Alecrim, A. D. O., Voltolini, G. B., Rezende, T. T., Menicucci-Netto, P. & Guimarães, R. J. (2019). Growth, anatomy and physiology of coffee plants intoxicated by the herbicide glyphosate, *Coffee Science*, 14(1), 76-82.

Cavalieri, S. D., Velini, E. D., Silva, F. M. L., São José, A. R., Andrade & G. J. M. (2012). Acúmulo de nutrientes e matéria seca na parte aérea de dois cultivares de soja RR sob efeito de formulações de glyphosate. *Planta daninha*, 30(2), 349-358.

Clairmont, K. B., Hagar, W. G., & Davis, E. A. (1986). Manganese toxicity to chlorophyll synthesis in tobacco callus. *Plant physiology*, 80(1), 291-293.

Dane, J. H., Topp, C. G. & Campbell, G. S. (2002). Particle size analysis. In *Methods of soil analysis. Part 4: Physical methods*. Soil Science Society of America Book Series, 5, 255-293.

Davis, J. G. (1996). Soil pH and magnesium effects on manganese toxicity in peanuts. *Journal of plant nutrition*, 19(3-4), 535-550.

DeGroote, K. V., McCartha, G. L., & Pollard, A. J. (2018). Interactions of the manganese hyperaccumulator *Phytolacca americana* L. with soil pH and phosphate. *Ecological research*, 33(4), 749-755.

Duke, S. O. & Reddy, K. N. (2018). Is mineral nutrition of glyphosate-resistant crops altered by glyphosate treatment? *Outlooks on Pest Management*, 29(5), 206-208.

Duke, S. O. (2018). The history and current status of glyphosate. *Pest management science*, 74(5), 1027-1034.

Duke, S. O., Lyndon, J., Koskinen, W. C., Moorman, T. B., Chaney, R. L. & Hammerschmidt, R. (2012a). Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. *Journal of agricultural and food chemistry*, 60, 10375-10397.

Duke, S. O., Reddy, K. N, Bu, K. & Cizdziel, J. V., (2012b). Effects of glyphosate on the mineral content of glyphosate resistant soybeans (*Glycine max*). *Journal of Agricultural and Food Chemistry*, 60, 6764–6771.

Eker, S., Ozturk, L., Yazici, A., Erenoglu, B., Romheld, V. & Cakmak, I. (2006). Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. *Journal of Agricultural and Food Chemistry*. 54, 10019–10025.

Ellis, J. M. & Griffin, J. L. (2002). Soybean (*Glycine max*) and cotton (*Gossypium hirsutum*) response to simulated drift of glyphosate and glufosinate. *Weed Technology,* 16, 580-586.

Europea (2014). U. Reglamento (UE) No 488/2014 de la Comision, de 12 de mayo de 2014, que modifica el Reglamento (CE) No 1881/2006 por lo que respecta al contenido máximo de cadmio en los productos alimenticios, 138. Diario Oficial de la Union Europea L, p. 13.

Fecht-Christoffers, M. M., Maier, P., Iwasaki, K., Braun, H. P., & Horst, W. J. (2007). The role of the leaf apoplast in manganese toxicity and tolerance in cowpea

(Vigna unguiculata L. Walp). In *The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions* (pp. 307-321). Springer, Dordrecht.

Fernando, D. R., & Lynch, J. P. (2015). Manganese phytotoxicity: new light on an old problem. *Annals of botany*, 116(3), 313-319.

Fitzgibbon, J. & Braymer, H. D. (1988). Phosphate starvation induces uptake of glyphosate by *Pseudomonas sp.* strain PG2982. *Applied and environmental microbiology*, 54:1886–1888.

SOURCEs, M. P. F. (2012). Intemperismo de rochas e minerais*. Pedologia-Fundamentos.* Viçosa, MG: Sociedade Brasileira de Ciência do Solo, 171-205.

França, A. C., Freitas, M. A. M., D'Antonino, L., Fialho, C. M. T., Silva, A. A., Reis, M. R. & Ronchi, C. P. (2010). Teores de nutrientes em cultivares de café arábica submetidos à deriva de glyphosate. *Planta daninha*, 28(4), 877-885.

Frigo, C., Magri, E., Barbosa, J. Z., Sarteretto, L. M., Araujo, E. M., Prior, S. A., & Motta, A. C. V. (2020). Influence of roadways on heavy metal content in soils and yerba mate tissue in southern Brazil. *Management of Environmental Quality: An International Journal,* 31 No. 6, pp. 1477-1495. https://doi.org/10.1108/MEQ-10-2019-0219.

Gatiboni, L. C., Da Silva, W. C., Mumbach, G. L., Schmitt, D. E., Iochims, D. A., Stahl, J., & Vargas, C. O. (2020). Use of exchangeable and nonexchangeable forms of calcium, magnesium, and potassium in soils without fertilization after successive cultivations with *Pinus taeda* in southern Brazil. *Journal of Soils and Sediments*, 20(2), 665-674.

Gaw, S. K., Kim, N. D., Northcott, G. L., Wilkins, A. L., & Robinson, G. (2008). Uptake of ΣDDT, arsenic, cadmium, copper, and lead by lettuce and radish grown in contaminated horticultural soils. *Journal of agricultural and food chemistry*, 56(15), 6584-6593.

Gee, G.W. & Or, D. (2002). 2.4 Particle-size analysis. *Methods of soil analysis: Part 4 physical methods*, 5, 255-293.

Gerhardt, M. (2013). História ambiental da erva-mate. Tese (Doutorado em História) – Universidade Federal de Santa Catarina, Florianópolis, 290 f.

Gravena, R., Filho, R. V., Alves, P. L. C., Mazzafera, P. & Gravena, A. R. (2012). Glyphosate has low toxicity to citrus plants growing in the field. *Canadian Journal of Plant Science*, 92(1), 119-127.

Grotz, N. & Guerinot, M. L. (2006). Molecular aspects of Cu, Fe and Zn homeostasis in plants. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1763(7), 595-608.

Gu, M., Chen, A., Sun, S., Xu, G. (2016). Complex regulation of plant phosphate transporters and the gap between molecular mechanisms and practical application: what is missing? *Molecular Plant*, 9:396–416.

Gupta, D. K., Chatterjee, S., Datta, S., Veer, V. & Walther, C. (2014). *Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals*. Chemosphere, 108, 134-144.

Hamid, Y., Tang, L., Sohail, M. I., Cao, X., Hussain, B., Aziz, M. Z., Usman, M., He, Z. & Yang, X. (2019). An explanation of soil amendments to reduce cadmium phytoavailability and transfer to food chain. Science of The Total Environment, 660, 80-96.. https:// doi.org/10.1016/j.scitotenv.2018.12.419.

Han, W. Y., Zhao, F. J., Shi, Y. Z., Ma, L. F. & Ruan, J. Y. (2006). Scale and causes of lead contamination in Chinese tea. *Environmental Pollution*, 139(1), 125-132.

Heinrichs, R. & Malavolta, E., 2001. Composição mineral do produto comercial da erva-mate (*Ilex paraguariensis* St. Hil.). *Ciência Rural*, 31(5), 781-785.

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., Jarvis, A. (2005). Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology: A Journal of the Royal Meteorological Society*. 25, 1965–1978. https://doi.org/10.1002/joc.1276.

Holmgren, G. G. S., Meyer, M. W., Chaney, R. L. & Daniels, R. B. (1993). Cadmium, lead, zinc, copper, and nickel in agricultural soils of the United States of America. *Journal of Environmental Quality*, 22 (2), 335–348.

Hothorn, T., Hornik, K. & Zeileis, A. (2006). Unbiased recursive partitioning: a conditional inference framework. *Journal of Computational and Graphical statistics*. Stat. 15 (3), 651–674.

Huang, Y. L., Yang, S., Long, G. X., Zhao, Z. K., Li, X. F., Gu, M. H., 2016. Manganese toxicity in sugarcane plantlets grown on acidic soils of southern China. *PLoS One,* 11 (3), e0148956.

Inmetro - Instituto Nacional de Metrologia and Normalização e Qualidade Industrial. (2020). DOQ-CGCRE-008, Orientações sobre validação de métodos de ensaios químicos.

Jin, C. W., Zheng, S. J., He, Y. F., Zhou, G. D. & Zhou, Z. X. (2005). Lead contamination in tea garden soils and factors affecting its bioavailability. *Chemosphere*, 59, 1151–1159.

Joint FAO/WHO Expert Committee on Food Additives. (2010). Joint FAO/WHO Expert Committee on Food Additives Seventy-third Meeting. World Health Organization, Geneva.

Junior, J. L., Moraes, M. F., Cabral, C. P. & Malavolta, E. (2008). Influência genotípica na absorção e na toxidez de manganês em soja. *Revista Brasileira de Ciências do Solo*, 32 (1), 173–181.

Kabata-Pendias, A. (2010). Trace elements in soils and plants, 4th ed. CRC Press, Pulawy, Poland.

Kaiser, H.F. & Rice, J. (1974). Little jiffy, mark IV. *Educational and psychological measurement*, 34 (1), 111–117.

Kidd, P., Mench, M., Alvarez-Lopez, V., Bert, V., Dimitriou, I., Friesl-Hanl, W., Herzig, R., Janssen, J. O., Kolbas, A., Muller, I., Neu, S., Renella, G., Ruttens, A., Vangronsveld, J. & Puschenreiter, M. (2015). Agronomic practices for improving gentle remediation of trace element-contaminated soils. *International journal of phytoremediation*, 17 (11), 1005–1037.

Kumar, A., Ghosh, A., Mondal, D., Ghosh, R. & Bandopadhyay, P. (2017). Bio-efficacy of ammonium salt of Glyphosate 71% SG for weed dynamics in tea (*Camellia sinensis* L.) and its effect on soil microflora. *International Journal of Current Microbiology and Applied Sciences*, 6, 2160-2170.

Lambers, H., Wright, I. J., Pereira, C. G., Bellingham, P. J., Bentley, L. P., Boonman, A., Cernusak, L. A., Foulds, W., Gleason, S. M., Gray, E. F., Hayes, P. E., Kooyman, R. M., Malhi, Y., Richardson, S. J., Shane, M. W., Staudinger, C., Stock, W. D., Swarts, N. D., Turner, B. L., Turner, J., Veneklaas, E., Wasaki, J., Westoby, M. & Xu, Y. (2020). Leaf manganese concentrations as a tool to assess belowground plant functioning in phosphorus-impoverished environments. *Plant and Soil*, 1-19.

Lane, M., Lorenz, N., Saxena, J., Ramsier, C. & Dick, R. P. (2012). The effect of glyphosate on soil microbial activity, microbial community structure, and soil potassium. *Pedobiologia*, 55(6), 335-342.

Leite, F. P., Novais, R. F., Silva, I. R., Barros, N. F., Neves, J. C. L., Medeiros, A. G. B., Ventrella, M. C., Villani, E. M. A. (2014). Manganese accumulation and its relation to eucalyptus shoot blight in the Vale do Rio Doce. *Revista Brasileira de Ciências do Solo,* 38(1), 193–204.

Linhares T. (1969). História econômica do mate. Livraria J. Olympio.

Ma, C., Liu, F. Y., Hu, B., Wei, M. B., Zhao, J. H., Zhang, K. & Zhang, H. Z. (2019). Direct evidence of lead contamination in wheat tissues from atmospheric deposition based on atmospheric deposition exposure contrast tests. *Ecotoxicological and Environtal Safety*, 185, 109688.

Magri, E., Gugelmin, E. K., Grabarski, F. A. P., Barbosa, J. Z., Auler, A. C., Wendling, I., Prior, S. A., Valduga, A. T., Motta, A. C. V., 2020. Manganese hyperaccumulation capacity of *Ilex paraguariensis* A. St. Hil. and occurrence of interveinal chlorosis induced by transient toxicity. Ecotoxicology and Environmental Safety, 203, 111010.

Magri, E., Valduga, A. T., Gonçalves, I. L., Barbosa, J. Z., de Oliveira Rabel, D., Menezes, I. M. N. R., Nascimento, P. A., Oliveira, A., Corrêa, R. S. & Motta, A. C. V. (2021). Cadmium and lead concentrations in yerba mate leaves from agroforestry and plantation systems: An international survey in South America. *Journal of Food Composition and Analysis*, 96, 103702.

Marcelo, M. C. A., Martins, C. A., Pozebon, D., Dressler, V. L. & Ferrão, M. F. (2014). Classification of yerba mate (*Ilex paraguariensis*) according to the country of origin based on element concentrations. *Microchemical Journal*, 117, 164–171.

Marcos, A., Fisher, A., Rea, G. & Hill, S. J. (1998). Preliminary study using trace element concentrations and a chemometrics approach to determine the geographical origin of tea. *Journal of Analytical Atomic Spectrometry*. 13 (6), 521–525.

Marques, A. C. (2014). As paisagens do mate e a conservação socioambiental: um estudo junto aos agricultores familiares do planalto norte catarinense (434 p. (Tese, curso de Doutorado em Meio Ambiente e Desenvolvimento da Universidade Federal do Paraná)).

Marques, R. & Motta, A. C. V. (2003). Análise química do solo para fins de fertilidade. In: Lima, M.R.de. (Ed.), *Manual de diagnóstico da fertilidade e manejo dos solos agrícolas, 2nd ed.* Departamento de Solos e Engenharia Agrícola, Curitiba, pp. 81–102.

Martınez-Villegas, N., Flores-V´elez, L. M. & Domınguez, O. (2004). Sorption of lead in soil as a function of pH: a study case in México. *Chemosphere* 57 (10), 1537–1542.

Mendoza, J. (2020). Latin America: yerba mate per capita consumption 2018, by country. Available in:https://www.statista.com/statistics/884925/consumption-yerba-mateper-capita-latin-america/#statisticContainerAccessed: September 10, 2020.

Milani, R. F., Silvestre, L. K., Morgano, M. A. & Cadore, S. (2019). Investigation of twelve trace elements in herbal tea commercialized in Brazil. *Journal of Trace Elements in Medicine and Biology*, 52, 111–117.

Millaleo, R., Reyes-Diaz, M., Ivanov, A. G., Mora, M. L. & Alberdi, M. (2010). Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition*, 10 (4), 470–481.

Miranda, M.; Vega-Gálvez, A.; Martínez, E. A.; López, J.; Marín, R.; Aranda, M. & Fuentes, F. Influence of contrasting environments on seed composition of two quinoa genotypes: nutritional and functional properties. *Chilean Journal of Agricultural Research*, 73(2):108-116, 2013.

Mitra, G. N. (2015). Regulation of Nutrient Uptake by Plants Gyanendra Nath Mitra A Biochemical and Molecular Approach. Springer.

Molitor, M., Dechamps, C., Gruber, W. & Meerts, P. (2005). Thlaspi caerulescens on nonmetalliferous soil in Luxembourg: ecological niche and genetic variation in mineral element composition. *New Phytol*ogist, 165 (2), 503–512.

Moreno-Jiménez, E., Fernandez, J. M., Puschenreiter, M., Williams, P. N. & Plaza, C. (2016). Availability and transfer to grain of As, Cd, Cu, Ni, Pb and Zn in a barley agri-system: impact of biochar, organic and mineral fertilizers. *Agriculture, Ecosystems & Environment*, 219, 171–178.

Motta, A. C. V., Barbosa, J. Z., Magri, E., Pedreira, G. Q., Santin, D., Prior, S. A., Consalter, R., Young, S. D., Broadley, M. R. & Benedetti, E. L. (2020). Elemental composition of yerba mate (*Ilex paraguariensis* A. St.-Hil.) under low input systems of southern Brazil. *Science of the Total Environment,* 736, 139637.

Munira, S., Farenhorst, A., Flaten, D. & Grant, C. (2016). Phosphate fertilizer impacts on glyphosate sorption by soil. *Chemosphere*, 53: 471-477.

Neumann, G., Kohls, S., Landsberg, E., Stock-Oliveira Souza, K., Yamada, T. & Romheld, V. (2006). Relevance of glyphosate transfer to non-target plants via the rhizosphere. *Journal of Plant Diseases and Protection*. 20: 963-969.

Oliva, E. V., Reissmann, C. B., Gaiad, S., Oliveira, E. B. & Sturion, J. A., 2014. Composição nutricional de procedências e progênies de erva-mate (*Ilex paraguariensis* St. Hil.) cultivadas em latossolo vermelho distrófico. *Ciência Florestal*, 24(4), 793–805.

Oliveira, Y. M. M., & Rotta, E. (1985). Área de distribuição natural de erva-mate (*Ilex paraguariensis* St. Hil.). In: S*eminário sobre atualidades e perspectivas florestais*, 10., 1983, Curitiba. Silvicultura da erva-mate (*Ilex paraguariensis*): anais... Curitiba: EMBRAPA-CNPF, 1985. p. 17-36.

Onwuka, B. & Mang, B. (2018). Effects of soil temperature on some soil properties and plant growth. *Scholarly Journal of Agricultural Science*, Res, 8, 34-37.

Pardinho, R. B., Vecchia, P. D., Alves, C. M., Pimentel, N., Gazzana, D., Bolzan, R. C. & Flores, E. M. (2020). *Ilex paraguariensis* exposition to As and Cd in a closed soilless system. *Chemosphere*, 258, 127284.

Pauletti, V. & Motta, A. C. V. (2017). (Coord.). Manual de Adubação e Calagem: Para o Estado do Paraná. *Curitiba: Sociedade Brasileira de Ciência do Solo - Núcleo Estadual do Paraná*. 482 p.

Penn, C. J. & Camberato, J. J., 2019. A critical review on soil chemical processes that control how soil pH affects phosphorus availability to plants. *Agriculture*, 9(6), 120.

Pereira, F. C. M., Tayengwa, R., Alves, P. L. D. C. A. & Peer, W. A. (2019). Phosphate Status Affects Phosphate Transporter Expression and Glyphosate Uptake and Transport in Grand Eucalyptus (*Eucalyptus grandis*). *Weed Science*, 67(1), 29-40.

PFA. (2003). Prevention of Food Adulteration Act of India, 1954 and Rules. *Eastern Book Company*, Lucknow, India.

Plante, A. F. & Parton, W. J. (2007). The dynamics of soil organic matter and nutrient cycling. In *Soil microbiology, ecology and biochemistry* (pp. 433-467). Academic Press.

Poletto, I., Muniz, M. F. B., Ceconi, D. E. & Blume, E. (2011). Influência da aplicação de NPK e calcário sobre o crescimento da erva-mate, severidade da podridão-de-raízes e população fúngica do solo. *Ciência Florestal*, 21 (3), 429–444.

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rabel, D. O., Motta, A. C. V., Barbosa, J. Z., Melo, V. F. & Prior, S. A., 2018. Depth distribution of exchangeable aluminum in acid soils: a study from subtropical Brazil. *Acta Scientiarum,* 40, e39320.

Rana, S. S., Kumar, R., Kumar, S., Sharma, N. & Badiyala, D. (2020). Evaluation of glyphosate against weeds and phytotoxicity, productivity and soil quality parameters in tea (*Camellia sinensis* O. kuntze). *Journal of Research in Weed Science*, 3(1), 90-100.

Reddy, K. N., Rimando, A. M., Duke, S. O. (2004). Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate-resistant soybean. *Journal of Agricultural and Food Chemistry*, 52, 5139–5143.

Reeves, R. D., Van Der Ent, A. & Baker, A. J. (2018). Global distribution and ecology of hyperaccumulator plants. In: *Van Der Ent,* A., Echevarria, G., Baker, A.J.M., Morel, J.L. (Ed.), Agromining: Farming for Metals. Springer, pp. 75–92.

Reissmann, C. B. & Carneiro, C. (2004). Crescimento e composição química de erva-mate (*Ilex paraguariensis* St. Hil.), transcorridos oito anos de calagem. *Floresta,* 34(3), 381–386.

Reissmann, C. B., Radomski, M. I. & Quadros, R. M. B. (1999). Chemical composition of *Ilex paraguariensis* St. Hil. under different management conditions in seven localities of Paraná State. *Brazilian Archives of Biology and Technology,* 42(2), 187–194.

Reitz, R., Klein, R. M., & Rus, A. (1988). *Projeto madeira do Rio Grande do Sul: termo de referencia: estudo da materia prima: levantamento das especies florestais nativas com possibilidade de incremento e desenvolvimento*. Sudesul.

Rizwan, M., Ali, S., Adrees, M., Ibrahim, M., Tsang, D. C., Rehman, M. Z., Zahir, Z. A., Rinklebe, J., Tack, F. M. & Ok, Y. S. (2017). A critical review on effects, tolerance mechanisms and management of cadmium in vegetables. *Chemosphere*, 182, 90–105.

Rizwan, M., Ali, S., Hussain, A., Ali, Q., Shakoor, M. B., Zia-ur-Rehman, M., Farid, M. & Asma, M. (2017). Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. *Chemosphere,* 187, 35–42. https://doi.org/10.1016/j.chemosphere.2017.08.071.

Roberts, T. L. (2014). Cadmium and phosphorous fertilizers: the issues and the science. *Procedia Engineering,* 83, 52–59.

Rodrigues, J. J., Worsham, A. D. & Corbin, F. T. (1982). Exudation of glyphosate from wheat (*Triticum aestivum*) plants and its effects on interplanted corn (*Zea mays*) and soybeans (*Glycine max*). *Weed Science*, 316-320.

Rosolem, C. A., Andrade, G. J. M. D., Lisboa, I. P. & Zoca, S. M. (2010). Manganese uptake and redistribution in soybean as affected by glyphosate. *Revista Brasileira de Ciência do Solo*, 34(6), 1915-1922.

Sadana, U. S., Kusum, L. & Claassen, N. (2002). Manganese efficiency of wheat cultivars as related to root growth and internal manganese requirement. *Journal of Plant Nutrition*. 25, 2677–2688.

Santin, D., Benedetti, E. L. & Reissmann, C. B. (2015). Nutrição e recomendação de adubação e calcário para a cultura da erva-mate. In: Wendling, I., Santin, D. (Eds.), *Propagação e nutrição de erva-mate*, pp. 99–195. Brasília: Embrapa.

Santin, D., Benedetti, E. L., Bastos, M. C., Kaseker, J. F., Reissmann, C. B., Brondani, G. E. & de Barros, N. F. (2013). Crescimento e nutrição de erva-mate influenciados pela adubação nitrogenada, fosfatada e potássica. *Ciência Florestal*, 23(2), 363-375.

Santin, D., Benedetti, E. L., Kaseker, J. F., Bastos, M. C., Reissmann, C. B., Wendling, I. & Barros, N. F. D. (2013). Nutrição e crescimento da erva-mate submetida à calagem. *Ciência Florestal*, 23(1), 55-66.

Santos, L. D. T., Santos, J. B., Ferreira, F. A., Oliveira, J. A., Bentivenha, S. & Machado, A.F.L. (2008). Exsudação radicular de glyphosate por *Brachiaria decumbens* e seus efeitos em plantas de eucalipto. *Planta Daninha*, 26(2), 369-374.

Santos, L. M. G. D., Neto, V., Alves, S., Iozzi, G. & Jacob, S. D. C. (2017). Arsenic, cadmium and lead concentrations in Yerba mate commercialized in Southern Brazil by inductively coupled plasma mass spectrometry. *Ciência Rural*, 47 (12).

Schmidt, S. B., Jensen, P. E. & Husted, S. (2016). Manganese deficiency in plants: the impact on photosystem II. *Trends Plant Science*, 21(7), 622–632.

Schmite, B. D. F. P., Bitobrovec, A., Hacke, A. C. M., Pereira, R. P., Los Weinert, P. & dos Anjos, V. E. (2019). In vitro bioaccessibility of Al, Cu, Cd, and Pb following simulated gastro-intestinal digestion and total content of these metals in different Brazilian brands of yerba mate tea. *Food Chemistry*, 281, 285–293.

Seenivasan, S., Anderson, T. A. & Muraleedharan, N. (2016). Heavy metal content in tea soils and their distribution in different parts of tea plants, *Camellia sinensis* (L). O. Kuntze. *Environmental monitoring and assessment*, 188 (7), 428.

Senger, C. C. D., Sanchez, L. M. B., Pires, M. B. G. & Kaminski, J. (1997). Teores minerais em pastagens do Rio Grande do Sul. II. Sódio, enxofre, zinco, cobre, ferro e manganês. *Pesquisa Agropecuária Brasileira*, 32(1), 101-108.

Serra, A. A., Nuttens, A., Larvor, V., Renault, D., Couée, I., Sulmon, C. & Gouesbet, G. (2013). Low environmentally relevant levels of bioactive xenobiotics and associated degradation products cause cryptic perturbations of metabolism and molecular stress responses in Arabidopsis thaliana. *Journal of Experimental Botany,* 64, 2753–2766.

Shao, J. F., Yamaji, N., Shen, R. F. & Ma, J. F. (2017). The key to Mn homeostasis in plants: regulation of Mn transporters. *Trends Plant Science*, 22(3), 215–224.

Silva, D. M. da, SOURCE, N. dos. S. da, Souza, K. R. D. de, Rodrigues-Brandão, I., Libeck, I. T. & Alves, J. D. (2017). Relationship between manganese toxicity and waterlogging tolerance in *Zea mays* L. cv. Saracura. *Acta Scientiarum. Agronomy*. 39(1), 75–82.

Silva, L. D. S., Gatiboni, L., Anghinoni, I. & Souza, R. (2016). Manual de calagem e adubação: Para os estados do Rio Grande do Sul e Santa Catarina. *Sociedade Brasileira de Ciência do Solo - Núcleo Regional Sul*, Comissão de Química e Fertilidade do Solo. Porto Alegre, 376 p.

Silvana, V. M., Carlos, F. J., Lucía, A. C., Natalia, A. & Marta, C. (2020). Colonization dynamics of arbuscular mycorrhizal fungi (AMF) in *Ilex paraguariensis* crops: Seasonality and influence of management practices. *Journal of King Saud University – Science*, 32(1), 183-188.

Siman, A., Cradock, F. W. & Hudson, A. W. (1974). The development of manganese toxicity in pasture legumes under extreme climatic conditions. *Plant Soil,* 41(1), 129–140.

Škrbić, B. & Čupić, S. (2005). Toxic and essential elements in soft wheat grain cultivated in Serbia. *European Food Research and Technology*, 221 (3), 361–366.

Škrbić, B. & Cvejanov, J. (2011). The enrichment of wheat cookies with high-oleic sunflower seed and hull-less barley flour: impact on nutritional composition, content of heavy elements and physical properties. *Food Chemestry*, 124 (4), 1416–1422.

Stafilov, T., Škrbić, B., Klánová, J., Čupr, P., Holoubek, I., Kočov, M. & Đurišić-Mladenović, N. (2011). Chemometric assessment of the semivolatile organic contaminants content in the atmosphere of the selected sites in the Republic of Macedonia. *Journal of chemometrics*, 25 (5), 262–274.

Subrahmanyam, D. & Rathore, V. S. (2000). Influence of manganese toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings. *Photosynthetica*, 38(3), 449–453.

Taiz, L., Zeiger, E., Møller, I. M. & Murphy, A. (2017). *Fisiologia e desenvolvimento vegetal.* Artmed Editora.

Tesfamariam, T., Bott, S., Cakmak, I., Römheld, V. & Neumann, G. (2009). Glyphosate in the rhizosphere - Role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants. *European journal of agronomy*, 31, 126-132.

Tong, M., Gao, W., Jiao, W., Zhou, J., Li, Y., He, L. & Hou, R. (2017). Uptake, translocation, metabolism, and distribution of glyphosate in nontarget tea plant (*Camellia sinensis* L.). *Journal of agricultural and food chemistry*, 65(35), 7638-7646. doi:10.1021/acs.jafc.7b02474.

Toppel, F. V., Junior, A. M., Motta, A. C. V., Frigo, C., Magri, E. & Barbosa, J. Z. (2018). Soil chemical attributes and their influence on elemental composition of yerba mate leaves. *Floresta*, 48(3), 425-434.

Troadec, M. B., Ward, D. M., Lo, E., Kaplan, J. & De Domenico, I. (2010). Induction of FPN1 transcription by MTF-1 reveals a role for ferroportin in transition metal efflux. *Blood, The Journal of the American Society of Hematology*, 116 (22), 4657–4664.

Tsushida, T. & Takeo, T. (1977). Zinc, copper, lead and cadmium contents in green tea. *Journal of the Science of Food and Agriculture*, 28 (3), 255–258.

Turner B. L. & Blackwell M. S. A. (2013). Isolating the influence of pH on the amounts and forms of soil organic phosphorus. *European Journal of Soil Science*, 64:249–259. https://doi.org/10.1111/ejss.12026

Usepa, E. (1995). Method 3051: Microwave assisted acid digestion of sediments, sludges, soils, and oils. *Test methods for evaluating solid waste*, pp 1-30.

Valduga, A. T., Gonçalves, I. L. & Magri, E., (2019). Analysis of the presence of toxic metals in yerba mate samples: a case study from South Brazil. *Water Air Soil Pollut*. 230 (7), 153.

Valduga, A. T., Gonçalves, I. L., Magri, E. & Finzer, J. R. D. (2019). Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. *Food Research International*. Int. 120, 478–503.

Van der Ent, A., Baker, A. J., Reeves, R. D., Pollard, A. J. & Schat, H. (2013). Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil. 362(1), 319-334.

Van der Ent, A., Ocenar, A., Tisserand, R., Sugau, J. B., Echevarria, G., Erskine, P. D. (2019). Herbarium X-ray fluorescence screening for nickel, cobalt and manganese hyperaccumulator plants in the flora of Sabah (Malaysia, Borneo Island). *Journal of Geochemical Exploration*, 202, 49-58.

Vieira, M. A., Rovaris, A. A., Maraschin, M., De Simas, K. N., Pagliosa, C. M., Podestá, R., Amboni, R. D. M. C, Barreto, P. L. M. & Amante, E. R. (2008). Chemical characterization of candy made of erva-mate (*Ilex paraguariensis* A. St. Hil.) residue. *Journal of agricultural and food chemistry*, 56(12), 4637-4642.

Wang, Q., Xie, Z. & Li, F. (2015). Using ensemble models to identify and apportion heavy metal pollution sources in agricultural soils on a local scale. *Environmental Pollution*, 206, 227–235.

Wendling, I., Santin, D., Nagaoka, R. & Sturion, J. A. (2017). BRS BLD Aupaba e BRS BLD Yari: cultivares clonais de erva-mate para produção de massa foliar de sabor suave. *Embrapa Florestas-Comunicado Técnico* (INFOTECA-E).

Werkenthin, M., Kluge, B. & Wessolek, G. (2014). Metals in European roadside soils and soil solution–a review. *Environmental Pollution*. 189, 98–110.

Whitley, E. & Ball, J. (2002). Statistics review 6: nonparametric methods. *Critical Care*, 6 (6), 509.

Xue, S. G., Chen, Y. X., Reeves, R. D., Baker, A. J., Lin, Q., Fernando, D. R. (2004). Manganese uptake and accumulation by the hyperaccumulator plant *Phytolacca acinosa* Roxb. (Phytolaccaceae). *Environmental Pollution*, 131 (3), 393–399.

Yamada, T. & Castro, P. R. D. C. (2007). Efeitos do glifosato nas plantas: implicações fisiológicas e agronômicas. *Informações Agronômicas,* 119, 1-32.

Yang, Y., Chang, A. C., Wang, M., Chen, W. & Peng, C. (2018). Assessing cadmium exposure risks of vegetables with plant uptake factor and soil property. *Environmental Pollution*, 238, 263–269.

Yaylalı-Abanuz, G. & Tüysüz, N. (2009). Heavy metal contamination of soils and tea plants in the eastern Black Sea region, NE Turkey. *Environmental Earth Science*, 59 (1), 131.

Zablotowicz, R. M. & Reddy, K. N. (2004). Impact of glyphosate on the Bradyrhizobium japonicum symbiosis with glyphosate-resistant transgenic soybean. *Journal of Environmental Quality*, 33, 825-831.

Zablotowicz, R. M. & Reddy, K. N. (2007). Nitrogenase activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean. *Crop Protection*, 26, 370-376.

Zanão Júnior, L. A., SOURCEs, R. L. F., Neves, J. C. L., Korndörfer, G. H. & Ávila, V. T. (2010). Rice grown in nutrient solution with doses of manganese and silicon. *Revista Brasileira de Ciências do Solo*, 34 (5), 1629–1639.

Zeng, F., Ali, S., Zhang, H., Ouyang, Y., Qiu, B., Wu, F. & Zhang, G. (2011). The influence of pH and organic matter content in paddy soil on heavy metal availability and their uptake by rice plants. *Environmental Pollution*, 159 (1), 84–91.

Zengin, F. (2013). Biochemical and physiological effect of excess manganese (Mn) in bean (*Phaseolus vulgaris* L. cv. Strike). *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, Sci. 83 (4), 651–657.

Zhao, H., Wu, L., Chai, T., Zhang, Y., Tan, J. & Ma, S. (2012). The effects of copper, manganese and zinc on plant growth and elemental accumulation in the manganese-hyperaccumulator *Phytolacca americana*. *Journal of Plant Physiology*. 169 (13), 1243–1252.

Zhao, H., Zhang, S. & Zhang, Z. (2017). Relationship between multi-element composition in tea leaves and in provenance soils for geographical traceability. *Food Control,* 76, 82–87.

Zheng, H., Li, J. L., Li, H. H., Hu, G. C. & Li, H. S. (2014). Analysis of trace metals and perfluorinated compounds in 43 representative tea products from South China. *Journal of Food Science*, 79 (6), 1123–1129.

Zobiole, L. H. S., Kremer, R. J., Oliveira, R. S. J. & Constantin, J. (2012). Glyphosate effects on photosynthesis, nutrient accumulation, and nodulation in glyphosate-resistant soybean. *Journal of Plant Nutrition and Soil Science*, 175, 319–330.

Zobiole, L. H. S., Kremer, R. J., Oliveira, R. S. J., Constantin, J. & Oliveira, R. S. (2011). Glyphosate affects chlorophyll, nodulation and nutrient accumulation of “second generation” glyphosate-resistant soybean (*Glycine max* L.). *Pesticide Biochemistry and Physiology,* 99, 53–60.

Zobiole, L. H. S., Oliveira, R. S., Kremer, R. J., Constantin, J., Yamada, T., Castro, C., Oliveira, F. A. & Oliveira, A. (2010). Effect of glyphosate on symbiotic N_2 fixation and nickel concentration in glyphosate-resistant soybeans. *Applied Soil Ecology*, 44, 176–180.